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Ethnobiology and Population Ecology of Neotropical Palms

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Ethnobiology and Population Ecology of Neotropical Palms

by

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Dedication

This dissertation is dedicated to my mother

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Ethnobiology and Population Ecology of Neotropical Palms

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Palms provide essential resources for local and indigenous human populations and frugivore species in the tropics. Many of those frugivores are important seed dispersers of palms, but are themselves hunted by humans for food. The interactions between palms, humans, and frugivores provide a unique system to examine how cultural and ecological components shape palm populations. In chapter 1, I examined the traditional knowledge and ecological implications behind the cultivation of palm-weevil larvae for food. The Joti people cultivated two species of weevil-larvae using two distinctive methods, which involved logging reproductive or non-reproductive palms. The cultivation of each weevil-larvae species thus had a direct and differential impact on palm populations. In chapter 2, I investigated how frugivores mediate interactions between two dominant and co-occuring palms in the Peruvian Amazonia-- *Attalea phalerata* and *Astrocaryum murumuru*. I found frugivores codispersed seeds of the two palm species, which contributed to aggregated patterns among their juveniles. Spatial patterns also suggested

aggregated heterospecific palms experienced lower density-dependent mortality than aggregated conspecifics and this likely contributes to the coexistence of the two palm species in their early life-history. These findings highlight the importance of dispersers to species coexistence and suggest over-hunting can lead to shifts away from species codominance. In chapter 3, I examined the contribution of seed dispersal, distance-and density-dependent mortality to the spatial patterns of *A. phalerata* population. Using microsatellite-based parentage analysis, I found high levels of seed movement mediated by frugivore dispersers. Seedlings however remained spatially aggregated with maternal trees. Spatial disassociations between older palm offspring and their maternal trees and between older maternal siblings indicate the importance distance- and density-dependent mortality in shaping the spatial patterns of palm trees in the post-recruitment phase. These results highlight the importance of seed-dispersers to the survival of *A. phalerata* recruits to maturity and warn of potentially severe impacts of hunting on palm populations. In chapter 4, we characterized 14 microsatellite loci for *A. phalerata* that were used in the parentage analysis of chapter 3. These loci amplified reliably and were sufficiently polymorphic and will be useful for future studies addressing population-level questions for this species.

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Introduction

Palms are considered the quintessential trees of the tropics (Henderson et al. 1995). Their ecological and cultural importance in tropical forest ecosystems reflects their ubiquity throughout the tropics. Among ethnobotanists, palms are considered the "tree of life" to the local communities that depend on its material and resource for their livelihoods; to the ecologists they are considered "keystone" species for the frugivore community in tropical rainforests because of the critical food resources they provide (Terborgh 1986). The influence of palms on forest communities raises the possibility of the far-reaching cascading impacts and negative feedbacks that will result from population decline or extinction. Palm populations worldwide are threatened directly by over-exploitation and deforestation and indirectly through activities that decrease the local population of their frugivore disperser mutualists. Effective conservation and management strategies for palms can therefore benefit from investigations aimed at understanding how specific activities of human and non-human animals that contribute to or hinder the processes that maintain the persistence of palm populations.

I began by investigating the traditional knowledge and ecological practices of the Joti people relating palm-weevil cultivation and their implications for palm populations (Chapter 1). Although palm-weevil cultivation has a long history and is widely practiced throughout indigenous communities of the tropics (Defoliart 1995), the intricacies behind the knowledge and practice of this activity remains under-investigated and the implications to palm populations associated with the logging activities for cultivating these weevils are unknown. Using ethnographic and quantitative assessments, I found the Joti possessed an intimate knowledge of palm and weevil interactions. The Joti selectively used *Oenocarpus bacaba* palms for weevil cultivation because its tissues were

most successful in attracting palm adults to oviposit and provided the optimum medium for larvae growth. I also discovered that the impacts of palm weevil cultivation to palm population depended on the species of weevil larvae cultivated since the Joti selectively logged reproductive or post reproductive palms for cultivating the larvae of *Rhinostomus barbirostris* but pre-reproductive ones for *Rhynchophorus palmarum*. The implications of weevil cultivation to the regeneration of *O. bacaba* palms thus depended on complex interactions between the preferred species of weevil larvae eaten and frequencies at which each was cultivated.

Preliminary findings on the significant reduction in palm seed dispersal near at Joti communities, which corresponded with hunting activities, provided the fodder for the subsequent chapters of my dissertation to investigate the how dispersal contributes to palm species coexistence and recruitment patterns. *Attalea phalerata* and *Astrocaryum murumuru* are two dominant and co-occurring palms of the Peruvian Amazon. Both species have fruits of strikingly similar morphology and share an overlapping community of frugivore dispersers. We therefore tested the hypothesis that seed dispersal and caching behaviors of frugivore promote the codispersal of the seeds of the two palm species and generate spatial associations between them (Chapter 2). Using seed tagging experiments and spatial analyses, we found support for both hypothesis and additionally showed that spatial aggregation between species had less impact on density-dependent mortality than spatial aggregation within species. These findings highlighted the importance of frugivore dispersers to the recruitment patterns of palms and mediating the interactions between palm species.

Frugivore dispersers also contribute critically to population recruitment patterns and genetic diversity. Using field, spatial and molecular approaches, I investigated how dispersal, seed predation, distance and density-dependence interacts to influence

recruitment patterns and spatial genetic structure in a population of *A. phalerata* (Chapter 3). Microsatellite-based parentage analysis allowed us to infer individual offspring dispersal and recruitment from parents and enabled us to rigorously test the influence of distance and density predation and mortality in palm populations. We found frugivores facilitated high levels of movement within and outside of the study plot. Frugivores also promoted the aggregation of recruits near non-parent adults. Despite these dispersal activities, we found offspring were aggregated near parents. Seed predation experiments indicated that predator satiation and/or caching behaviors may contribute to the escape from predation and promote aggregated patterns between offspring and their parents or non-parent adults. There was also a trend for offspring to disassociate from parents, non-parent adults, and siblings, confirming the influence of distance and density-dependent mortality. The combined interactions of dispersal, distance and density-dependent mortality were also important in contributing to the weaker spatial genetic structure among recruits in older cohorts. Parentage analysis also allowed us to detect significant skew and high attrition in number of parents contributing the population of recruits near reproductive age, raising questions about the mechanisms driving this pattern. Collectively, our findings show the importance of frugivores to the spatial and genetic structure of our palm population and provide a foundation for understanding implications of hunting on palm populations.

Finally in chapter 4, we describe characterization of 14 microsatellite markers for *Attalea phalerata* that facilitated the parentage analysis of chapter 3. These markers amplified reliably and were sufficiently polymorphic for our current study and will also be useful in future studies addressing questions in the dispersal or pollination ecology of *A. phalerata* palms.

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Chapter 1: The importance of traditional ecological knowledge for palm-weevil cultivation in the Venezuelan Amazon

Abstract: Entomophagy is widespread among indigenous people, promoting the gathering of traditional ecological knowledge of insect life histories and plant-insect interactions. In the Amazon, the cultivation of palm weevil larvae (*Rhynchophorus palmarum* and *Rhinostomus barbirostris*) for food provides an important supplement to the diets of many indigenous people. This study conducted with the Jotï people from Venezuelan Amazonia examined their traditional ecological knowledge (TEK) of palm (*Oenocarpus bacaba*) and weevil interactions and how they have adapted their TEK to cultivation practices. We found that Jotï traditional ecological knowledge was congruent with scientific findings of weevil natural history and palm and weevil interactions. The Jotï have applied their traditional ecological knowledge to optimize returns on palm weevil cultivation. They manipulate palms to induce changes in the resource-partitioning and competition that occurs naturally between weevil species, thereby increasing harvests of their preferred species of weevil. This analysis identifies potential research directions that may provide solutions to agricultural problems such as palm weevil infestations in palm plantations. We conclude that understanding and preserving traditional ecological knowledge and practices is important for organisms such as palm weevils that rarely have been studied in their natural forest settings.

1.1 INTRODUCTION

Entomophagy, the use of insects as food by humans, has long captured the interest of biologists and anthropologists. It is practiced by cultures worldwide, but to varying degrees by indigenous and westernized people (Choo 2007; Defoliart 1999; Ramos-Elorduy 1997). Across the tropics, at least 2000 insect species are eaten as food (Ramos-Elordy 2005). Within the biologically hyper-diverse Amazon basin region, indigenous groups consume at least 209 species of insects (Paoletti and Dufour 2005). Insect foods are considered delicacies in this region, but they also provide protein, fat, and vitamin supplements to the diet (Bukkens 1997; Cerda et al. 2001; Dufour 1987).

While many species of weevils are consumed as food, *Rhynchophorus* (Coleoptera: Curculionidae) weevils are the most widely used in the world (Defoliart 1995). In Amazonia, they are one of the few insect foods that are managed or cultivated by many indigenous groups, including the Tukanoan, Barí, Hiwi, and Yukpa (Beckerman 1977; Cerda et al. 2001; Dufour 1987; Ruddle 1973). The life history and behavior of *Rhynchophorus* weevils facilitate human manipulation and cultivation. Adult weevils are gregarious. They congregate to mate on palm tissues and each female can lay up to hundreds of eggs. The larvae that emerge burrow into palm tissues where they remain until maturity (Howard et al. 2001). Therefore, large numbers of developing larvae can easily be harvested from a single palm source. By cultivating weevil larvae, people have control over the location and timing of this larvae food supply. They can also assess the progress of larvae development and plan their harvest at a time when the larvae are optimally large.

The general process of cultivating palm weevil larvae has been described for a number of indigenous groups (see papers in Defoliart 1995). Palms are cut down to

attract the adult weevils and a few months later the larvae are harvest. What remains unexplored is the traditional ecological knowledge (TEK) associated with weevil cultivation. As forest inhabitants, indigenous people have numerous opportunities to learn about the natural history of insects and to accumulate this knowledge over the generations. We therefore expect traditional indigenous groups that frequently practice weevil cultivation to possess a significant body of TEK on palm and weevil interactions (Zent and Zent 2004a).

Studies show the value of integrating TEK in conservation efforts, restoration ecology, resource management, environmental studies, and population monitoring (Bart 2006; Berkes et al. 2000; Drew 2005; Fraser et al. 2006; Gilchrist et al. 2005). Palm weevil cultivation provides an ideal system to document and evaluate TEK in the realm of plant and insect interactions. The current body of scientific research on palm and palm weevil interactions has largely focused on studies in palm plantations because palm weevil infestations have lead to significant economic losses (e.g., Faleiro et al. 2003; Oehlschlager et al. 2002). As a consequence, palm weevil ecology and behavior in natural forest settings are not well-known (although see Eberhard 1983). The traditional knowledge of forest dwellers may provide an important complement to the scientific understanding of interactions between palms and weevils in their natural settings.

This study documents the TEK of palm weevil cultivation for the Jotï, a traditional and semi-nomadic group of the Venezuelan Amazon. We begin by examining the Jotï's TEK of weevil life history and how they have integrated this knowledge into developing a successful system for cultivating weevils. We then compare TEK with scientific knowledge, and address areas of Jotï TEK meriting further investigations. Lastly, we highlight how human activities can influence the natural interactions and resource-partitioning between plants and insects.

1.2 MATERIALS AND METHODS

The first author conducted the fieldwork for this study between June to August of 2005 and 2006, in the Sierra Maigualida region, close to the state borders of Amazonas and Bolivar, Venezuela. Because the region is inaccessible, an in-depth ethnology of the Jotï people was only recently documented (see Zent and Zent 2004a, 2004c). We conducted our research in three of the approximately 20 distinct Jotï communities in Venezuela: San Jose de Kayama, Caño Iguana, and Caño Majagua (Figure 1). We estimate a population of 300 at Kayama, 165 at Caño Iguana, and 25 at Caño Majagua (Zent and Zent 2004b).

To examine Jotï's traditional ecological knowledge of weevil ecology and behavior, we compiled information from 18 informants during weevil cultivation expeditions and conducted semi-structured interviews. The estimated ages of consultants ranged from 25 to 55 years, and three were female. Semi-structured interviews were either carried out directly with consultants who spoke Spanish or with the assistance of a Jotï translator when consultants only spoke the native language. To identify weevils that the Jotï cultivated for food, we used the identification keys of Wattanapongsiri (1966)

1.3 RESULTS

TEK of weevil life history and behavior.—The Jotï actively cultivate two species of edible palm weevil – *Rhynchophorus palmarum* (L.) and *Rhinostomus barbirostris*

(Fab.) (Figure 2). Data from this study and those collected by S. Zent and E. L. Zent (unpublished data), suggest that larvae of *R. barbirostris* are cultivated more frequently than *R. palmarum*. According to the Jotï, the species taste different; *R. barbirostris* larvae have a richer flavor. The Jotï refer to the larvae of *R. palmarum* as ***uli badebodï*** and those of *R. barbirostris* as ***jani badebodï***. The words "***uli***" and "***jani***" mean large and small respectively, and describe the relative size of the two species of larvae ***Badebodï*** refers to the *Oenocarpus bacaba* palms in which the weevil larvae are cultivated. The eggs, pupae, and adult of stages of both weevil species respectively are called ***ïë***, ***jojo jadï***, and ***wajlilijka***. Every Jotï consultant correctly distinguished between the adults of the two species, and 15 of the 18 also correctly distinguished males from females. To distinguish sexes, the consultants most frequently noted the presence (in males) or absence (in females) of bristles on the rostrums and the smaller bodies of the males.

The Jotï also provided a general description of weevil mating. Consultants indicated that for both weevil species, odors from the cut palm tissues initially attract male and female weevils to congregate and mate. Males initiate mating by mounting the backs (dorsi) of females. Female weevils then drill holes in the palm trunks to oviposit. Consultants emphasized that female weevils appear to be selective about where their eggs are deposited, with *R. palmarum* females preferring to deposit eggs where the inner palm tissues are exposed, while *R. barbirostris* females prefer the surface of trunks, close to internodal scars, and areas with few other eggs. Consultants believe that after mating, *R. palmarum* adults disperse to other cultivation sites in search of fresh palms, whereas many *R. barbirostris* weevils die, because the Jotï frequently find dead *R. barbirostris* adults at cultivation sites.

Our consultants described that after emerging from the eggs, the weevil larvae feed on the inner palm tissues and tunnel deeper into the palm trunk. They observed that

R. palmarum larvae also burrow out from the trunks into the soil for short periods and then re-enter the trunk. When approaching the pupae stage, *R. barbirostris* larvae begin to tunnel their way towards the trunk surface to facilitate subsequent adult emergence. The larvae then carve a thin circular cap near the surface of the trunk , which facilitates their emergence after transforming in the the adult stage. *R. palmarum* larvae, on the other hand, migrate to areas of exposed trunk tissue (e.g., where cuts were previously made) to facilitate their emergence as adults. The Jotï note that at the pupal stage, *R. palmarum* larvae create cocoons using materials from palm tissues, but *R. barbirostris* larvae do not form cocoons (Figures 3 and 4). The Jotï describe the period of metamorphosis when the weevil larvae transform to the pupal and adult stages, literally as the "changing of skin."

TEK of the palm weevil cultivation process.— The Jotï reported that under natural forest settings they find and collect weevil larvae in several palm species including *Attalea maripa* (Aubl.) Mart., *Bactris* spp., *Euterpe oleraceae* (Mart.), *Mauritia flexuosa* (L.f.), and *Oenocarpus bacaba* (Mart.). However, when they cultivate the weevil larvae, the Jotï preferentially use *O. bacaba,* as noted in Zent and Zent (2004a). The Jotï consider *O. bacaba* ideal for weevil cultivation presumably because of the large number of larvae they can harvest and the flavor that the palm tissues give to the larvae. Within naturally fallen *O. bacaba* palms, relatively low numbers of *R. palmarum* larvae are found near the base of trunks where palm tissue is exposed, while *R. barbirostris* larvae are more numerous, colonizing the length of the intact trunks. If a cultivator wishes to harvest predominantly *R. barbirostris* larvae, a felled trunk can remain intact. However, further manipulation of the trunk is required if the cultivator chooses to cultivate *R. palmarum* larvae.

Using this knowledge of palm weevil life history, the Jotï adapt their cultivation practices to the specific traits of *R. barbirostris* and *R. palmarum*. They consider four important factors to ensure optimal larvae harvests: 1) when to cultivate – rainy season or dry season, 2) the age, size, and species of available palms, 3) the need to cut into palm trunks to facilitate larvae entry, and 4) when to harvest the larvae. The second and third factors are directed at controlling the species of weevil larvae cultivated.

According to the Jotï, exposed inner palm tissues more strongly attract *R. palmarum* than *R. barbirostris* weevils to mate and lay eggs. *R. palmarum* adults feed on the inner tissues and the females prefer to deposit eggs in exposed inner palm tissues than on the surface of an intact palm trunk. By making wedge-shape cuts into palm trunks after they are cut down, the Jotï facilitate the colonization of *R. palmarum* larvae in these trunks, which would otherwise be dominated by *R. barbirostris* larvae. The Jotï usually make two wedge-shaped cuts along the palm trunks using axes or machetes, cutting deep enough to penetrate to the pith. The first cut is on average 1.58 m (n = 7, SE = 0.23) from the palm crown, and the second is on average 1.54 m (n = 8, SE = 0.12) away from the first. They do not cut near the base of the palm trunk because those tissues are tough and provide poor conditions for larvae development. The Jotï consultants estimate *R. palmarum* weevils arrive at a palm trunk within 2 to 24 hours after the inner tissues of the palm trunks were exposed (Figure 5). *R. barbirostris* weevils, on the other hand, colonize the intact palm trunks 1 to 2 days after palms were cut down (Figure 6). The Jotï explain that the presence of the early-arriving *R. palmarum* adults and larvae "spoil" the palm for the late-arriving *R. barbirostris*. *R. palmarum* larvae presumably degrade and/or deplete the palm such that conditions are unsuitable for *R. barbirostris* larvae**.** Consequently, palms felled and cut to expose the inner palm tissues yield predominantly

R. palmarum larvae rather than *R. barbirostris*. So the Jotï decide whether to leave the trunks entire or cut into them depending on which weevil species they want to harvest.

The Jotï also control which weevil species will infest a palm trunk by selecting a particular age and size of palm. Relatively young, short palms, in their pre-reproductive stages are considered ideal for cultivating *R. palmarum* larvae because the odor of the young palm's inner tissues are highly attractive to the adults and the soft inner tissues are ideal for larvae feeding and development. On the other hand, according to the Jotï, *R. barbirostris* weevils are attracted to mature and reproductive palms, which provide the ideal medium for their larvae development. Using the height of a palm as a proxy for its age, we found significant differences in the heights of palms cut down for cultivating the two weevil species (*t*-test with unequal variances: $t_{21} = -5.39$, n = 24, $P < .001$); those cut down for *R. palmarum* were on average shorter (mean = 5.92 m, n = 9, SE = 0.45) than those for *R. barbirostris* (11.23 m, n = 15, SE = 0.89).

After felling and if necessary, cutting into the trunk, the Jotï leave the palms on the forest floor until the larvae are ready to be harvested. They may return occasionally before the harvest to check on the larvae's development. Individual cultivators use a variety of methods to discern whether the larvae are ready for harvest, such as counting the days or lunar cycles (i.e., 29.5 days), or examining the color of sawdust expelled from the entry holes larvae create as they tunnel through the palm trunks. Our consultants indicated that in the early stages, the sawdust is whitish, but subsequently, when the weevil larvae are ideal for harvesting, the sawdust turns a darker orange-yellow color. Our observations show that these methods accurately track larvae development of the two species. We accompanied the Jotï on several forays and found that their harvests often coincided with a time when relatively few larvae had already pupated or adults had eclosed. The time from felling a palm to harvest differed significantly between *R.*

palmarum and *R. barbirostris* (t-test with unequal variances: t_{18} = -7.56, n = 14, P < .01). *R. palmarum* larvae developed faster with a mean time-to-harvest of 2.25 months (n = 8, SE = 0.37) while *R. barbirostris*, were slower with a mean of 4 months (n = 6, SE = 0.45).

The Jotï generally harvest *R. palmarum* larvae from the entire palm trunk, but only harvest *R. barbirostris* larvae from the upper half of the trunks (mean proportion of trunk harvested=43.7%, n = 6, SE = 8.8) (Figure 7). Larvae harvest commences from the upper portion of the trunk, just below the palm crown. The Jotï split trunks in half with an axe and extricate the larvae with their bare hands and knives. When we requested that consultants split open the unharvested portion of a trunk in order to assess its contents, we noticed fewer and smaller larvae than in the harvested portions. Since we could not collect data on changes in larva size along the length of the trunk, we indirectly inferred this using the size of larval tunnel holes. Larval tunnel diameters sampled from every meter-section between 4 to 7 m from the base of the trunk were significantly different ($F_{3, 33}$ = 2.89, p < .001). Tunnels were larger toward the palm crown, suggesting that larvae were larger as well. The Jotï harvested the relatively few pupae of both species when encountered, and although some also collected freshly eclosed *R. barbirostris* adults with soft exoskeletons, in general, the Jotï released newly eclosed adults so they could produce more weevil larvae.

During the harvesting process, we observed children and teenagers consuming raw *R. barbirostris* larvae, but not *R. palmarum* larvae. The Jotï believe that eating raw *R. palmarum* larvae induces stomach upsets. The harvested larvae were taken back to the homesteads and cooked in soups, smoked or roasted over fires. According to unpublished data from S. Zent and E. L. Zent, each Jotï household harvested on average

2424 grams (n = 17, SE = 550) of *R. barbirostris* larvae, or 2120 grams (n = 5, SE = 341) of *R. palmarum* larvae from each harvesting foray.

The Jotï cultivate weevil larvae primarily from September to January, the end of the rainy season and the beginning of the dry season. During the peak rainy period, from June to August, heavy and frequent rain deters or prevents adult weevils from seeking out palms, resulting in reduced larvae harvests. The Jotï also believe that weevil eggs are susceptible to damage during downpours. We observed that the rainy season may also promote fungal attack that kill larvae. Conditions during the peak of the dry season, approximately February and March, also inhibit larval development. According to the Jotï, felled palms desiccate rapidly at this time, so weevils are not attracted to lay eggs in them. Those Jotï who successfully cultivate weevils in the dry season reported that they cut down palms growing under the canopy of tall trees where shade maintained sufficiently high levels of humidity to prevent the felled palms from drying. Alternatively, cultivators cover the felled trunks with palm leaves to prevent them from desiccating.

Observations of other weevil species.—We found adults of three weevil species that are known to attack palms besides *Rhynchophorus palmarum* and *Rhinostomus barbirostris*: *Metamasius hemipterus* (L.), *Dynamis borassi* (F.), and *Homalinatus* sp. We set out traps baited with fresh sugar cane, Combolure ® (ChemTica International SA, San Jose, Costa Rica), and ethyl-acetate on trails at the Kayama study site. We recovered 249 *R. palmarum* adults but only three *D. borassi* and one *Homalinatus* sp. The number of *M. hemipterus* adults, was not tabulated; however many individuals were trapped. The larvae of *Dynamis* borassi and *Homalinatus* sp. were not encountered among the samples of *R. palmarum* and *R. barbirostris* larvae harvested by the Jotï and identified by the first author. However, since it was not possible to identify every harvested larvae, we cannot

rule out the possibility that small numbers of *D. borassi*, *M. hemipterus*, and/or *Homalinatus* sp. larvae were included in the harvest.

Conserving palms for weevil cultivation—The Jotï's folk beliefs may encourage palm conservation by instilling a fear of retribution for misuse of resources. According to the Jotï, excessive exploitation of forest resources angers spiritual beings, ***jkyo aemodï*** (see Zent 2005), who then inflict ailments such as fevers, stomach pains, and even death in extreme cases on the perpetrators. In the context of palm weevil cultivation, a number of cultivators explicitly rotated every 2-3 years between *Oenocarpus bacaba* groves, ***bate jkwa***, in their efforts to avoid depleting palm populations. Despite their belief system and individual efforts to conserve palms, the Jotï note a significant decline in *O. bacaba* populations. According to a 40-year resident of Caño Iguana, *O. bacaba* palms were abundant within 2 km of settlements when the consultant first moved there and when the Jotï population consisted of small communities of 5 to 35 highly mobile people. However, the increased population at Caño Iguana (currently estimated at 165) and a corresponding increase in felling palm trees to cultivate weevil larvae have depleted *O. bacaba* palms close to Jotï settlements. As a result, the Jotï presently travel between four and twenty hours from their homesteads to encounter suitable palms for cultivating larvae. Assuming an average normal walking speed of 3.6 km/h, this suggests a distance of 14.4 km to 72 km.

Our Jotï consultants at Caño Iguana also believe that this depletion of *O. bacaba* trees has reduced palm weevil populations. They have observed that at present relatively few weevils are attracted to palms that were cut for weevil cultivation and that weevils are taking longer to colonize cut down trunks. In spite of the scarcity of *O. bacaba* palms near settlements, the Jotï do not cultivate them, although they do cultivate another palm, *Bactris gasipaes* Kunth. Unsuitable environmental conditions near settlements, such light

gaps or poor soil quality, may limit seedling recruitment. Jotï consultants report that *O. bacaba* seeds that germinate in garbage heaps usually do not survive beyond the seedling stage, presumably due to desiccation.

1.4 DISCUSSION

The Jotï's TEK of palm weevil life history and behavior coincide with several findings in Hagley (1965), Eberhard (1983) and Howard et al. (2001). Observations that weevil species preferentially lay eggs on different parts of the palm trunk may be result from weevils selecting sites that increase chances of offspring survival (Renwick 1989). *R. barbirostris* females prefer to lay eggs away from previously oviposited sites and this may be a strategy to avoid intra-specific competition among offspring that is documented for other weevil taxa (Heard 1995; Messina and Renwick 1985). *R. palmarum* females appear to target oviposition in exposed inner palm tissues, which may facilitate larvae access to food.

The dead *R. barbirostris* adults at cultivation sites noted by our Jotï consultants may result from intra-specific competition among male adults, since males are known to engage in intense battles for females (Eberhard 1983). However, these weevils are nocturnally active and the cultivation sites were far from settlements, so we could not confirm that weevil mortality was associated with male competition. This study also allows us to clarify conflicting reports of whether *R. barbirostris* larvae construct cocoons before the pupae stage (Bondar 1940; Howard et al. 2001; Vaurie 1968; Wolcott 1933). The Jotï's TEK and the first author's observations confirm that they do not.

The Jotïs also have a good understanding of palm weevil feeding preferences and nutritional ecology . The Jotïs prefer *Oenocarpus bacaba* palms as host for cultivating weevil larvae, suggesting that weevils find some species of palms more attractive than others. Studies of weevil infestation rates at palm plantations provide additional indirect evidence for species-specific attraction of weevils to palms. For example, Giblin-Davis et al. (1996) found that coconut palms (*Cocos nucifera* L.) are more susceptible to weevil attack than African oil palms (*Elaeis guineensis* Jacq.).

The species of palm also influences larval development, with larval size dependent on the quality and quantity of palm tissue (Howard et al. 2001). Feeding experiments showed that *R. palmarum* larvae reared on *Mauritia flexuosa* weighed 9.6 grams on average, while those reared on *Jessenia bataua* (Martius) Burret, weighed 4.6 grams, over a two-fold difference (Cerda et al. 2001). Even within individual palm trunks, there exist variations in the hardness of the palm tissues or quality of food available to the larvae. In addition, Eberhard (1983) and Bondar (1940) showed that larval density and size are apparently influenced by differences in the nutritional quality of inner palm tissues along trunks. Larvae were smaller and less densely concentrated in the 3 m closest to the base. The Jotï harvest *R. barbirostris* only from the upper trunk segments, which indicates that they recognize this spatial variation in the quality of larvae along individual trunks.

The mechanisms for inter- and intraspecific variation in palm attraction and suitability for weevil larval development are unclear and require further investigation. While palm weevils exhibit a broad niche breadth and use many palm species as hosts (Howard et al. 2001), the Jotï's success using *Oenocarpus bacaba* suggests that palm weevil generalists may favor and perform better in specific palm species. Singer (1982) documented that other phytophagous insects vary in their degree of preference to a range

of available hosts. Since palm weevils are notorious pests of palms, a better understanding of how host palm quality and host preference influence weevil population dynamics will likely shed light on the epidemiology of weevil infestation.

Applying their TEK of palm weevil feeding preferences, the Jotïs have discovered a way to influence the natural partitioning of resource between palm weevil-species and the species of weevil larvae cultivated. Our research shows that the Jotï control which weevil species colonizes a palm by modifying the trunk to attract specific weevils and facilitate oviposition and larval growth; exposing the inner palm tissues favors *Rhynchophorus palmarum* larvae on a resource generally dominated by *Rhinostomus barbirostris* larvae. Studies show that fermented exudates from the inner palm tissues are highly attractive to *R. palmarum* adult weevils and that palm weevils preferentially aggregate, mate and oviposit in the wounds of palms (Faleiro et al. 2002; Giblin-Davis and Howard 1989; Giblin-Davis et al. 1996; Hagley 1965; Murphy and Briscoe 1999; Wattanapongsiri 1966; Weissling and Giblin-Davis 1994). However, other mechanisms may also be involved in causing on palm weevil larvae to dominate over another.

We need additional research to understand how peoples' manipulation alters species-specific larvae dominance. Some possible mechanisms are pheromone avoidance or inter-specific larval predation/competition. For example, when multiple species of scolytid beetles are sympatric, the pheromones of one species deter the arrival of competing species, resulting in different beetle species colonizing different regions of the trunk (Ayres et al. 2001). In addition, inter-specific larval competition among beetles can lead to significant mortality in subordinate competitors. Predation by large cerambyscid beetle larvae, for example, causes 76% of the mortality of a subordinate bark beetle's larvae on the same tree (Dodds et al. 2001).

The Jotï's control of the weevil species they cultivate may have nutritional implications. Additional studies will also help us determine the criteria the Jotï use to decide which species of weevil to cultivate and in what proportions. Although we documented that more *R. barbirostris* larvae are cultivated than *R. palmarum*, we do not know the nutritional contribution of each species to the Jotï's diet. We documented that taste preference is an important factor, and we hypothesize that the success of cultivating each species and the availability of other insect and non-insect food resources may also influence this decision.

Our investigations indicated that the Jotï's were informed about seasonal fluctuations in palm weevil populations. The Jotï's TEK of the optimal timing of weevil cultivation, at the end of the rainy season and into the beginning of the dry season, points to their awareness of seasonal changes in weevil population and activity. We have no scientific data on the population dynamics of *R. barbirostris*, but studies of *R. palmarum* show that weevil abundance is seasonally driven by rainfall patterns. *R. palmarum* weevil populations tend to peak toward the end of the rainy season in Trinidad (Hagley 1965) and during most of the dry season in Brazil, Costa Rica, and Honduras (Chinchilla et al. 1990; Schuiling and Van Dinther 1981). Thus, the Jotï cultivate larvae when adult weevil populations and activity are likely to be relatively high. In addition, the Jotï avoid cultivating larvae during the peaks of the dry and wet seasons, because the rapid deterioration of palm resources during these periods produces low weevil larvae returns. Oehlschlager and Gonzales (2001) have shown that fresh palm material, used to trap weevils in plantations, desiccated rapidly during the dry season and, decomposed rapidly in the wet season, becoming ineffective in attracting adults. Both the Jotï's TEK and scientific data indicate that the attractiveness of palm resource is influenced by seasonal conditions, which in turn determine successful larval colonization of the palms. This

information can be applied to the management of commercial palm harvests because harvesting palms during the peak dry season may minimize weevil larvae infestations.

Our study on the Jotï's *palm weevil cultivation further provided qualitative data on its potential effects on palm populations and forest community*. Historic palm abundance and distribution are difficult or impossible to collect when current palm populations are absent or low. This study of palm weevil cultivation allows us to infer that *Oenocarpus bacaba* palms were historically abundant in the Caño Iguana region. Population growth and increasingly sedentary lifestyles at this settlement combined with the destructive nature of palm weevil cultivation practices may have contributed to the local depletion of *O. bacaba* populations. Weevil cultivation may also influence the regeneration and recruitment of other plant species. Gaps generated from felling palms, for instance, may facilitate the establishment of shade intolerant seedlings. It is also conceivable that, as suggested by the Jotï, depleted palm populations have in turn reduced local population of palm weevils. The cascade effects of palm weevil cultivation on insect and plant populations will require further investigation for us to better understand the consequences of indigenous peoples' food acquisition on forest biodiversity and regeneration in Amazonia.

The current findings confirm the Jotï's extensive TEK related to the ecology of palm and weevil interactions, and supported by scientific literature, raises intriguing questions about palm weevils that merit further investigation. By combining Jotï's TEK with scientific findings, we may be able to better understand the ecology of palm weevils in their natural settings and the implications of anthropogenic activities on historic and present palm populations. Traditional ecological knowledge of plant and insect interactions remains an understudied area, and we hope that this case study serves to highlight the importance of examining the ecological implications of traditional

subsistence activities as well as the potential of TEK in complementing scientific research.

FIGURES

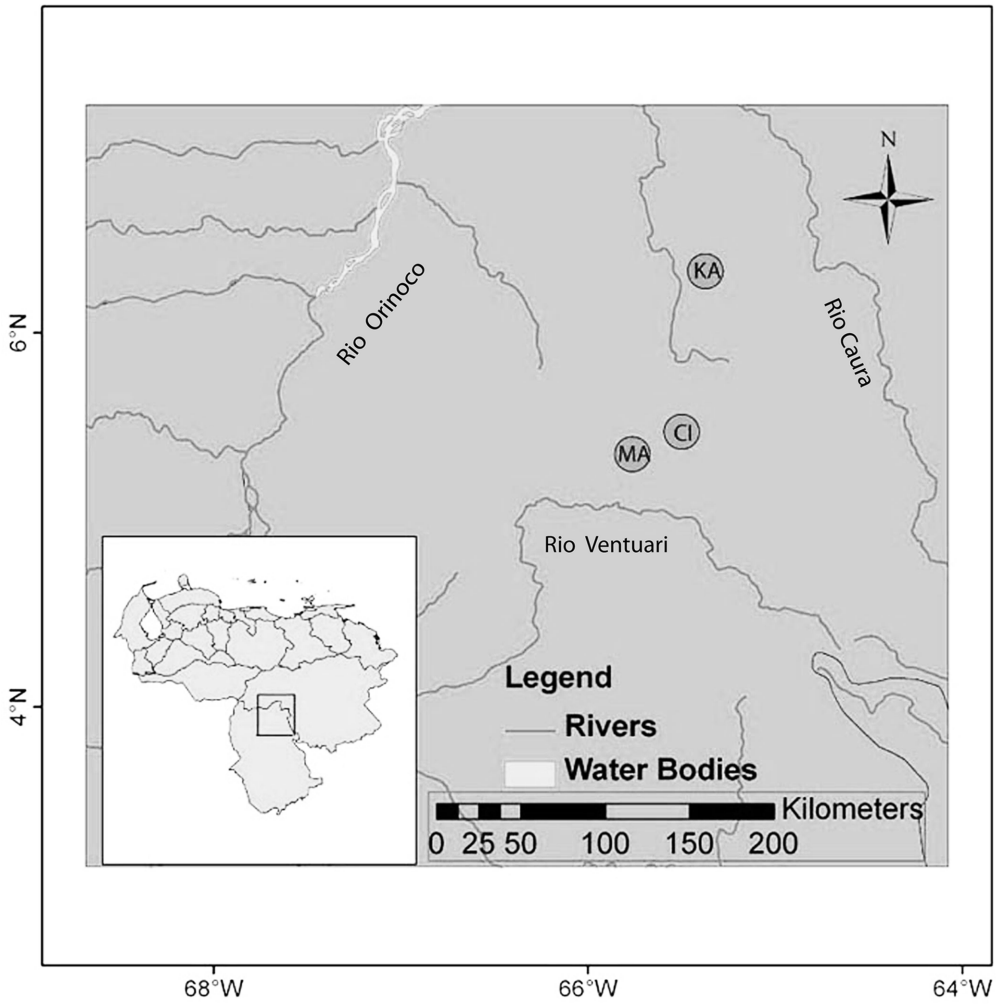

Figure 1.1: The three Jotï study sites in Venezuela – Caño Iguana (CI), Kayama (KA), and Majagua (MA).

Figure 1.2: Harvested larvae of *Rhinostomus barbirostris*.

Figure 1.3: *Rhinostomus barbristoris* larva, pupae, and adult (from top left counter-clockwise).

Figure 1.4: *Rhynchophorus palmarum* larva in cocoon, larvae, and pupa (from left to right).

Figure 1.5: *Rhynchophorus palmarum* adults in a cut created in the trunks of *Oenocarpus bacaba* palms and a larva harvested from the trunk (inset).

Figure 1.6: *Rhinostomus barbirostris* adult males.

Figure 1.7: *Oenocarpus bacaba* palm trunks showing portions of trunks that were harvested for larvae.

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Chapter 2: Seed dispersal and the spatial interactions of two dominant Neotropical palms of the Peruvian Amazon

Abstract: Patterns of seed dispersal are complex and affect not only the escape of seeds from predation but also the spatial association between plants. Increasing numbers of studies are finding that frugivores generate patchy patterns of seed dispersal involving multiple seed species. These findings have important consequences for the probability of recruitment success of patchily dispersed species. In this study we used seed tagging experiments and spatial analyses to investigate the hypothesis that shared frugivore seed dispersers of *Attalea phalerata* and *Astrocaryum murumuru* palms are responsible for codispersing palms seeds to similar sites and promoting spatial aggregation between the two palm species. We also investigated the implications of codispersal on the density-dependent interactions among heterospecific palm aggregates compared to conspecific palm aggregations. We found evidence of codispersal and support for spatial aggregation between seedling and juvenile individuals of the two palm species. Patterns of palm aggregation indicate weaker density-dependent mortality in heterospecific aggregates in the seedling and juvenile stages than conspecific aggregations, providing support for the benefits of plant-plant facilitation or "herd-protection" associated with heterospecific neighborhoods. Codispersal activities thus promote the coexistence of palms in the early life-history stages. We discuss possible effects of hunting and dispersal loss on the associations and interactions between the two palm species.

2.1 INTRODUCTION

One of the most important events affecting flowering plants is seed dispersal. Dispersal determines the patterns of seed movement and recruitment, which in turn affect the intensity at which individual offspring experience post-dispersal events such as predation and competition (Nathan and Muller-Landau 2000). The factors that influence seed dispersal dynamics are therefore critical in the trajectories of plant population growth and species coexistence in forest communities.

The majority of tree species in many tropical forests have fruits that are animal-dispersed (Foster and Janson 1985, Chazdon et al. 2008). The community of frugivores and the dispersal services they provide thus exert a strong influence on forest community dynamics. Seed dispersal behaviors of frugivores influence the patterns of aggregation among conspecific plant individuals and dispersal distances from reproductive trees (Seidler and Plotkin 2006, Muller-Landau et al. 2008). These patterns create the template within which plants interact with the distance- and density-dependent predators that facilitate species coexistence in the tropics (Janzen 1970, Connell 1971). Under the Janzen-Connell model, two kinds of predators – distance and density-dependent predators - work in tandem to regulate plant populations. Distance-dependent predators concentrate near adult trees; their impact on juvenile survival is thus inversely related to the distance from adults. Density-dependent predator on the other hand are predators that have an impact on the survival of juveniles that is inversely related to the distance between juveniles, in other words, whether juveniles are aggregated or dispersed. The overaching thesis of the Janzen-Connell model are that changes to the components that affect the survival probability of recruits will consequently determine the recruit patterns of juveniles away from adults. Frugivore-mediated seed dispersal away from parental tree

should lead to increased survival and recruitment probabilities for plants. However studies do not find consistent support this thesis (Hyatt et al. 2003). A major factor contributing to this discrepancy is the Janzen-Connell model's over simplification of the complexity of seed-dispersal particularly for animal-dispersed plants. Frugivores exhibit complex seed-dispersal behaviors and do not disperse fruits singly or in the leptokurtic or exponentially decreasing manners predicted in the model.

Frugivores display non-random behaviors that can lead to patchy patterns of seed dispersal, termed "contagious dispersal" (Schupp et al. 2002). Various examples of contagious seed dispersal have been documented in the tropics. Spider monkeys, for instance, defecate *Virola calophylla* seeds at their sleeping sites resulting in conspecific seedling clumps forming beneath these trees (Russo and Augspurger 2004) and tapirs are known to deposit large numbers of *Attalea maripa* palm seeds at tapir latrines (Fragoso 1997). Contagious dispersal of seeds involving more than one frugivore group and the seeds of multiple plant species have also been documented for bird-plant interactions (Schupp et al. 2002, Clark et al. 2004, Kwit et al. 2004). These examples are however far from a complete list of the potential networks of frugivore-plant interactions that promote contagious dispersal of seeds given the diffuse nature of frugivore mutualisms. Palms for instance provide an excellent system for investigating possible species groups associated with contagious dispersal. Palms species are often dominant in tropical forests, frequently have sympatric distributions, and many share frugivore dispersers (Henderson et al. 1995). Scatter-hoarding rodents for instance are one of the most important disperses of mammal-dispersed palms (Forget 1991, Hoch and Adler 1997, Wright and Duber 2001). Moreover many sympatric palm species exhibit similar fruit morphology that increase the probability of frugivores dispersing their seed to similar sites. Despite these factors, it is surprising that studies have not looked into possible patterns of contagious

dispersal in palms even though they have been model taxa for numerous seed-dispersal studies testing the Janzen-Connell model (e.g., (Wright and Duber 2001, Wyatt and Silman 2004)

Contagious dispersal has interesting implications for the predictions of Janzen-Connell model and species coexistence. First, contagious dispersal does not guarantee escape from seed predation if predators are also responding to the abundance of other seed species (Kwit et al. 2004). Second, contagious dispersal promotes spatial aggregation and density-dependent interactions among trees species that can have important implications for species coexistence (Clark et al. 2004). Studies show density-dependent mortality associated with crowding of heterospecific or conspecific trees affect over 80% of tropical trees species in sites in Panama and Malaysia (Peters 2003). Plant species that are contagiously dispersed may therefore experience higher levels of density-dependent mortality compared to other species. On the other hand, studies also indicate that heterospecific aggregations of tropical trees can help trees escape the detection of specialist predators, a phenomenon labeled as "herd-protection" (Wills et al. 1997). The herd-protection hypothesis suggests that heterospecific neighbor plants can decrease the risk of specifics specific predators detecting their plant host if these neighbors that are resistant to, or not affected by, such predators. Herd-protection can thus increase the survival probability trees if the benefits gained from escaping pest or predators outweigh the costs of resource competition with heterospecific neighbors (Peters 2003). Spatial analyses of tropical plant distributions provide a means to investigate these complex implications of contagious dispersal on the interactions among long-lived heterospecific trees. Spatial patterns of plant distribution provide important clues to the underlying processes impacting plant population and communities (Perry et al. 2002) because many

interactions between plants and the forest community leave spatial signatures (Wiegand and Moloney 2004).

In this study, we investigated the dispersal and recruitment patterns of two sympatric and dominant palm species of the Peruvian Amazon. Seeds of *Attalea phalerata* Mart. and *Astrocaryum murumuru* Mart. (Arecaceae: Cocoeae) are both dispersed by several frugivore species including scatter-hoarding rodents (*Agouti paca, Dasyprocta punctata, Sciurus* spp.), brown capuchin monkeys (*Cebus apella*), and tapirs (*Tapirus terrestris*) (Cintra 1997a, Cintra 1997b, Quiroga-Castro and Roldán 2001). Evidence from various studies also show that scatter-hoarding rodents exhibit non-random seed dispersal behaviors in (e.g., (Heaney and Thorington Jr 1978, Aliaga-Rossel et al. 2008). From the foregoing, we hypothesized that scatter-hoarding rodents disperse *Attalea phalerata* and *Astrocaryum murumuru* seeds to similar caching or feeding sites. If this hypothesis is true, we predict: 1) seedlings and juveniles from the two palms should exhibit spatial aggregation with one another, 2) dispersal activities and seedling abundance of the two palms species will correspond with the combined abundance of reproductive palm adults, 3) heterospecific aggregates of palms will experience weaker density-dependent mortality than conspecifics as predicted by the herd-protection hypothesis. We conclude by discussing the implications of our findings in light of hunting impacts and the loss of frugivore dispersers on *Attalea phalerata*.

2.2 METHODS

Overview of experimental design. We tagged and followed the dispersal of *Attalea phalerata* palm seeds to find evidence of contagious or patchy dispersal of palm seeds.

We mapped and censused all palm individuals in our study site in order to investigate the predictions that dispersal leads to spatial associations between the species. We then compared the patterns of aggregation within and between palm species of different size cohorts to investigate the patterns of density-dependence mortality over time and to test the predictions of herd-protection. In both analyses, we accounted for the effects of environmental heterogeneity that may influence our findings. Finally we used cluster analysis to detect hot spots of seed dispersal activities and the relationship with the abundance of reproductive palms.

Study species and site. This study was conducted in a 2.25 hectare plot (150 m x 150 m) at the Cocha Cashu Biology Field Station (CC) in Manu National Park Peru. Cocha Cashu is well-known for its "pristine" forest condition. The high diversity and density of large birds and mammals are considered to be representative of the forest fauna before the advent of exploitative hunting (Gentry and Terborgh 1990). *Attalea phalerata* and *Astrocaryum murumuru* are dominant canopy palms at Cocha Cashu. They produce morphologically similar fruits of comparable dimensions (Figs. 1 and 2) and are major fruit resources for several mammal frugivores including scatter-hoarding rodents (*Agouti paca, Dasyprocta punctata*), white-lipped peccaries (*Tayassu pecari*), and tapirs (*Tapirus terrestris*).

Seed dispersal. We tagged and followed the dispersal of a total of 181 intact fruits and 216 seeds of *Attalea phalerata* for three weeks. Seed were tagged using nylon-line and flagging tape attached to the end. We established 36 feeding stations that were evenly distributed throughout the study plot. Each station was given five (one had six) fruits and six seeds. We visited each station to look for dispersal activity on a daily basis and

conducted systematic searches of the entire study plot to recover fruits that were moved. At each location where fruits and seeds were moved but were not buried, we also searched within a 2-meter radius for the fruits and seeds of *Astrocaryum murumuru* or other plant species.

Palm census. We mapped all individuals of *Attalea phalerata* and *Astrocaryum murumuru* found within the 2.25 hectare plot (150 x 150 m). For each individual encountered, we documented its size using the longest leaf as a proxy. If palms had emergent trunks, we estimated the trunk height using a Suunto clinometer. We categorized palm population into four distinct stages - reproductive adults (A), juveniles with emergent trunk (AJ), juveniles (J), and seedlings (B). Seedlings and juveniles were distinguished by their leaf morphology. Seedlings possess entire leaves while juveniles have divided leaves. Palm juvenile heights varied widely between 0.5 m to 10.0 m and we subdivided them into three size categories J1 (less than 2.0 m), J2 (2.0 to 4.0 m), and J3 (greater than 4.0 m). These categories were selected so that sample sizes were comparable and were sufficiently large for the purpose of our analyses. Size classes are also not intended to reflect the absolute age of the palms but we assume they are a good approximation of relative age.

Spatial analyses. We conducted all spatial analyses with the software Programita using methods and recommendations of Wiegand and Moloney (2004). We applied the univariate g(r) and bivariate $g_{12}(r)$ pair correlation function to examine spatial associations between palm species as well as those among conspecifics. The pair correlation function was chosen over Ripley's K function statistics (Ripley 2004) for documenting second-order spatial characteristics because we are interested in detecting

small-scale associations arising from plant-plant interactions and identifying the distances at which these interactions occur.

The pair correlation function (Eqn 1) is similar to the K-function except that instead of a circle, it counts the number of points within a ring of radius *r* and width *dr* centered on an individual. Specifically in Equation 1, $R_i^w(r)$ represents a ring with radius *R* and width *w* and this ring is centered on the *i*th point of a pattern. Because the pair correlation function is not a cumulative distribution like the K-function, it allows for more precise detection of the specific distances of associations (Wiegand and Moloney 2004).

$$g_{12}^w(r) = \frac{\sum_{i=1}^{n_1} Point s_2\left[R_i^w(r)\right]}{\sum_{i=1}^{n_1} Area_2\left[R_i^w(r)\right]} \qquad (1)$$

We applied the numerical approach to estimate g(r). This approach involved dividing the study plot into a grid of cells. We selected a grid cell size of 0.5 m, which is greater than our original mapping resolution but sufficiently small to address our hypothesis within reasonable computational time. The numerical approach also circumvented problems associated with edge effect by generating a statistic that is derived only from points falling within the study plot (Wiegand and Moloney 2004).

We used Monte-Carlo simulation envelopes and the goodness-of-fit test (GOF) to assess our observed point pattern against the null models (see below). We rejected the null hypothesis when the observed data fell outside of the simulated envelopes and when the p-value for GOF was less than 0.05. Following (Wiegand et al. 2007b) and (Stoyan and Stoyan 1994), we used 99 simulations if the observed pattern had 300 or more points

or 999 simulations otherwise. The highest and lowest values of the 99 simulations were used for constructing envelopes while the 5th highest and lowest values were used for 999 simulations.

Spatial null models. In order to address the hypothesis and predictions of this study, we used three different spatial null-models. The null-model for complete spatial randomness (CSR) allowed us to detect any potential environmental heterogeneity (or first-order effects) that could lead to aggregated patterns of our palms. We then removed these first-order effects to detect spatial interactions between palms species and cohorts (i.e., second-order effects). In our tests for interactions between point patterns, we used the null-model of independence as a preliminary step to assess if two point patterns are independent. Rejection of the independence null-model indicates significant spatial interactions between the two point patterns (i.e., aggregation or repulsion between species or cohorts). For significant interactions between cohorts of different sizes, we further applied a third null model – the antecedent condition. This null-model allows us to test for attraction or repulsion between point patterns while accounting for the fact that one of the point patterns (i.e., older cohort) was established prior to the other (i.e., younger cohort). This allowed us to determine if seeds are preferentially dispersed to and recruiting near locations where palms have already previously existed.

Complete spatial randomness (CSR) is a commonly used null-model for tests of spatial interactions in point patterns. Under CSR, points in the study area W have a homogeneous intensity λ, are independent to one another, and follow a Poisson distribution with mean λW (Ripley 2004). CSR is an appropriate null model for testing spatial aggregation of points as a result of first-order effects such an environmental or habitat heterogeneity. Many natural systems display significant environmental or

topographical factors of first-order effects. It is therefore necessary to remove the effects of the environment on the spatial associations of point patterns in order to test for the second-order effects related to small-scale point associations such as plant-plant interactions (Wiegand and Moloney 2004, Wiegand et al. 2007b).

To detect first-order effects in our study plot, we used the pair correlation function g(r) and a null model of complete spatial randomness (CSR). If first-order effects are prevalent, g(r) will show significant large-scale clustering over a range of distances (i.e., virtual aggregation) and with intensities greater than λ (Wiegand and Moloney 2004). We also conducted a preliminary test for the prevalence of second order effects using the L-statistic (which is a transformation of the K statistics). A steeply increasing L(r) at small-scales confirms the prevalence of first-order effects (Wiegand and Moloney 2004).

Because first order effects were found in our system, we used the heterogeneous Poisson process (HPP) to account for these first-order effects. Instead of a homogeneous intensity of points, the heterogeneous Poisson process generate points for the null-model pattern using an intensity that varies with coordinates (x,y) and is a function $\lambda(x,y)$. To calculate the variable intensity function we use the Epanečnikov kernel (Stoyan and Stoyan 1994). Briefly, intensity is calculated using a circular moving window centered on (x,y) with radius or bandwidth R. Points falling within the circle are counted and weighted according to their distance *d* from the center. Selecting an appropriate R is important because it influences directly the estimates of intensity. We chose an R of 20 m based on the range of inter-plant interactions that have been documented in the Neotropics (Hubbell et al. 2001, Uriarte et al. 2004) and prior studies showing neutral interactions of species separated beyond this range (Wiegand et al. 2007a).

The independence model assumes that two populations are independent and is appropriate when the spatial location of two patterns were *a priori* the result of two

different processes (e.g., two species or cohorts) (Goreaud and Pélissier 2003). A rejection of the null model indicates that the location of one population is not independent of the other and supports further analysis using the antecedent model. To implement the test of independence null model, at each Monte-Carlo simulation, we generated new point processes comprised of two independent populations that have the same spatial structure as the original populations. This is done by conserving the spatial structure one population while randomly shifting the entire component pattern of the other to break their inter-dependence in a study area that is treated as a torus to facilitate the random shifting of the data points (Goreaud and Pélissier 2003)

The antecedent null model assumes that two independent processes created the two point patterns but pattern 2 was created after pattern 1 had existed. The null model is generated by holding the locations of one palm cohort fixed (pattern 1) while randomizing the other palm cohort (pattern 2) over the study area at an intensity that accounts for environmental heterogeneity. For our analyses, we held the larger cohorts fixed and randomized the smaller cohorts as we assumed larger cohorts were older and were present in the system prior to their small and younger counterparts. This allowed us to determine if seeds are preferentially dispersed to, and recruiting near, locations where palms already previously existed.

Cluster analysis of seedlings. We used the graph theoretic method of Plotkin et al. (2002) to detect the location of seedling clusters formed by the two palm species. Graphs are a system of nodes connected by links called edges. There are different types of nodes and these can range from landscapes to molecules while edges such as dispersal routes or chemical bonds represent the physical or qualitative link to connect these nodes. We first converted our spatial data of palms seedlings to a graph with seedlings representing

nodes and the displacement (or dispersal) distance *d* between seedlings representing the edges. We used the percolation threshold of the clustering probabilities (i.e., the probability of finding two seedlings in a cluster) to detect the scales at which non-random clustering in the seedlings occurs and to also detect the location of these clusters.

A single-linkage hierarchical clustering (HC) approach was used to generate clusters in our palm network with respect to *d*. For each *d* value, seedlings that are *d* or less apart were joined with edges and each of these groupings formed a cluster. We then calculated the average number of seedlings in all the clusters that formed for each *d*. This process was repeated for progressively larger values of *d* until all seedlings were joined in a single connected cluster. Once clustering of the palm network was established we determined the critical distance at which the clustering patterns of seedlings exhibit a percolation threshold, the transition from a poorly connected to a well-connected system Plotkin et al. (2002). This critical distance represents the non-random scale of aggregation for our seedlings and we used this value to determine the cluster locations of seedlings in our study plot. For each seedling cluster, we calculated the total number adults found within the cluster or within 10 m from any seedling on the periphery of the cluster. We used the *R* statistical software to fit linear model for the relationship between cluster-size (i.e., number of seedlings) and adult abundance.

2.3 RESULTS

Seed dispersal. We recovered tagged-fruits of *Attalea phalerata* at 14 sites where seeds of *Astrocaryum murumuru* were also deposited. Between one to four *Astrocaryum murumuru* seeds were found alongside our tagged *Attalea phalerata* fruits. We did not

find seeds or fruits from other plant species at these locations during the period of this study. Fruits and seeds from both palms species were found away from relatively open microhabitats, specifically under fallen logs, at the bases of tree trunks, and sites with dense under-story cover of fallen twigs and vines.

Palm census. Densities of *Astrocaryum murumuru* (0.071 per m^2) were 2.7 times higher than *Attalea phalerata* (0.026 per m^2) and the population of *Astrocaryum murumuru* consisted of a larger proportion of juveniles. A summary of the size frequency distribution of *Astrocaryum murumuru* and *Attalea phalerata* is given in (Table 2.1).

Spatial statistics. Spatial patterns of the entire population of *Attalea phalerata* and *Astrocaryum murumuru* indicate significant first-order effects. Results of the pair-correlation function showed virtual aggregation in the patterns for both palm species (Fig. 2.3). A steeply rising at L-function between 0-10 m indicated significant patterns of small-scale aggregation among conspecifics in the each palm population (Fig. 2.3).

Spatial evidence for associations between palm species. In the spatial associations of non-stemmed cohorts, our data indicated *Attalea phalerata* and *Astrocaryum murumuru* palms were more aggregated than expected by our null-models. These heterospecific aggregations involved palms primarily in the seedling through J2 cohorts (Table 3.2). Specifically, aggregated patterns were found between *Attalea phalerata* and *Astrocaryum murumuru* palms of the same cohorts (e.g., seedlings vs. seedlings) (Table 2.2, Fig. 2.5) as well as palms belonging to different cohorts (e.g., seedlings vs. J3) (Table 2.2, Fig. 2.4). In the latter case, we also found evidence from tests of the antecedent null-model for the attraction of smaller cohorts to larger

heterospecific and conspecific palms, suggesting directed seed dispersal near larger palm individuals or facilitation by larger palm individuals. In all the above cases (except 1), spatial aggregations occurred at small-scales ranging between 0-5 m.

We did not however find evidence of spatial aggregations between the stemmed cohorts of *Attalea phalerata* and *Astrocaryum murumuru*. Adults and stemmed-juvenile (AJ) were randomly spaced with respects to individuals in same-size cohorts and those in different-size cohorts. Adults and stemmed-juveniles were also either randomly or negatively associated with all other non-stemmed cohorts (Table 2.2).

Spatial evidence of density-dependence among heterospecific palms. Spatial patterns among same-size heterospecifics suggest relatively weaker density-dependent mortality over time compared to same-size conspecifics. *Attalea phalerata* and *Astrocaryum murumuru* palms were aggregated with one another from the seedling through to the J3 cohorts (Table 2.2, Fig. 2.5). This contrasts with the earlier loss of aggregation among conspecifics of the same cohorts (see below)(Table 2.3). We also found spatial patterns indicating weak density-dependence mortality for mix-cohort interactions between the two palm species (Table 2.2).

Spatial evidence of density-dependence among conspecific palms. Spatial data suggests density-dependent mortality occurs between conspecifics through time. Both palm species exhibited a general spatial trend of aggregation among individuals in smaller cohorts (Fig. 2.6, 2.7 and 2.8) that later gave way to negative or random patterns among individuals in the stemmed cohorts (A and AJ) (Table 2.3). Same-size conspecifics of *Attalea phalerata* palms in particular appear to experience more severe density-dependent interactions in their early life stages based the loss of spatial associations in the early cohorts (i.e. J1). For both palm species, spatial interactions between conspecifics of mix-cohorts (e.g., seedling and J1) show the attraction of smaller

cohorts to larger ones, indicating weaker density-dependence mortality among these groups (Table 2.3).

Palm cluster analysis. Cluster analysis of the seedlings of *Attalea phalerata* and *Astrocaryum murumuru* indicated an percolation threshold at a critical distance of 7.6 m. At this non-random scale of aggregation, we identified 15 large (i.e. >10) seedlings clusters and found significant associations between clusters size and the number of reproductive adults ($F_{1,13}$=27.73, p=0.001) (Fig. 2.9 and 2.10)

2.4 DISCUSSION

Evidence for codispersal. Our spatial and field data support the hypothesis that frugivore dispersers are dispersing seeds of both *Attalea phalerata* and *Astrocaryum murumuru* to similar locations, although not necessarily at the same time. In addition to finding seeds together, we also found *Attalea phalerata* and *Astrocaryum murumuru* seedlings were aggregated and showed that these aggregates did not result from competing factors such as the spatial associations among their adults or environmental heterogeneity. However, we did not find large numbers seeds of the two species together as expected in contagious dispersed seeds. We therefore refer to these dispersal activities as the codispersal of seeds rather than contagious dispersal of seeds as defined in Schupp et al. (2002).

At Manu and elsewhere, *Attalea phalerata* and *Astrocaryum murumuru* palm seeds are primarily scatter-hoarded by red squirrels (*Sciurus grantensis*) and agoutis (*Dasyprocta punctata*) (Glanz et al. 1982, Cintra 1997b) and we believe they are the

agents responsible for the codispersal of these palm seeds at the scale of our study. The locations where we recovered the palm seeds and the numbers we found corresponded with previous findings on the patterns of scatter-hoarding behaviors in rodents. Squirrels and agoutis frequently cache seeds near lianas, at the base of small trees, against buttress roots, or tree falls and these caches were generally small consisting of six or fewer seeds (Heaney and Thorington Jr 1978, Aliaga-Rossel et al. 2008). We argue that such non-random seed dispersal behaviors are promoting the codispersal of heterospecifics palms seeds even if the tree fruiting phenologies do not overlap. The fact that we recovered *Attalea phalerata* and *Astrocaryum murumuru* seeds in the same locations but at different times further indicates dispersers are returning to feeding or safe-sites at different times of the year. This finding is consistent with the behavior of agoutis moving between refuges near their sleeping sites and such refuges may be associated with locations where we recovered the palm seeds (Aliaga-Rossel et al. 2008)

Our spatial data also provide clues to the temporal and spatial patterns of codispersal. We found *Attalea phalerata* and *Astrocaryum murumuru* palms were not only aggregated in the seedling cohort but also at each progressively larger juvenile cohort. These patterns can be explained by the codispersal of heterospecific palm seeds over several fruiting seasons. In addition, the persistent spatial associations between smaller seedling or juvenile cohorts and larger heterospecific palms (e.g., seedlings and J2) further indicate frugivores are dispersing seeds into the same refuges or feeding sites over several seasons. This is tenable considering the life history of scatter-hoarding rodents. Available data suggest that red squirrels can live to about 9 years, while agoutis can live for 17 years (Aliaga–Rossel 2004, Milton et al. 2005). Although the actual life spans of these frugivores are likely to be shorter as a result of predation, many will likely live for several years. We therefore hypothesize that a subset of the extant community of

squirrels and agoutis at Cocha Cashu may have been responsible for generating the aggregates of heterospecific palms seedlings and juveniles.

Cluster analysis provided a novel method for us to detect the centers localized seed dispersal activity in our study plot using data from the recruitment patterns of seedlings. We found local dispersal activities i.e. cluster-size corresponded with the number of reproductive trees thus supporting second hypothesis and pointing to the importance of neighborhood heterospecific trees in attracting seed dispersers and facilitating seed dispersal as previously demonstrated in other systems (Carlo 2005, Carlo and Aukema 2005). Because scatter-hoarding rodents are known to locate their homes or refuges near key resources such as *Attalea phalerata* and *Astrocaryum murumuru* palms (Aliaga–Rossel 2004), these localized clusters of seedlings are likely associated with their sleeping or resting sites.

Codispersal: implications on seed predation and recruitment. The observed aggregation of *Attalea phalerata* and *Astrocaryum murumuru* seedlings suggest that codispersed seeds are successfully escaping seed predation and are recruiting into the seedling stages. Since the codispersal of the two species of palm seeds were associated with relatively few seeds (<6), seeds are more likely to escape detection from predators compared to seeds found under palms trees where seed densities are many fold higher (Cintra 1997a). Moreover, differences in the phenological patterns of the two palm species likely results in the dispersal of heterospecifics palms seeds to frugivore refuges or feeding sites at staggered time periods. This minimizes the synchronous accumulation of heterospecific seeds within a season and may therefore prevent high seed predation associated with apparent competition as documented in other studies (Clark et al. 2004). In terms of the dispersal activities on seedling survival, we hypothesize that the

associations of seedlings or smaller palm cohorts with larger individuals may facilitate the growth of seedlings. Studies have documented the benefits of physical associations with vegetative structures showing a significant number of seedlings escape mortality associated with the trampling activities of peccaries (Wyatt and Silman 2004)

Codispersal: implications on density-dependence mortality. The codispersal of heterospecific palm seeds and the aggregated patterns of seedlings have important consequences for probability of palm survival into the reproductive stages and their long-term coexistence. We infer the long-term impacts of codispersal on the density-dependent interactions among heterospecific seedling aggregation by following changes in the spatial associations of palm individuals in progressively older palm cohorts and comparing these patterns with the corresponding data from conspecifics.

In general, our spatial data suggest that *Attalea phalerata* and *Astrocaryum murumuru* respond similarly to the effects of density-dependent interactions. We found partial support for our third prediction - weaker density-dependent mortality among heterospecifics than conspecifics during the seedling and juvenile phases. These patterns suggest first that palms in heterospecific aggregates are benefitting from herd-protection and second that palms in same-size heterospecific aggregates experience less intense density-dependent mortality than those in conspecific aggregates. The codispersal of palms seeds thus appears to promote palm establishment at least in the early life-history phase. Such benefits gained from associating with heterospecific aggregates have also been shown elsewhere. Harms et al. (2000) found that tropical plant seedlings were more likely to establish near heterospecifics than conspecifics (Harms et al. 2000).

The benefits of associating with heterospecifics was however ultimately lost in the stemmed-cohorts of our palms. It is probable that during these stages, the costs of

competition for resources associated with the development and growth of trunks in these palms individuals out-weighed the benefits gained from associating with heterospecifics. These findings show that the codispersal of palms seeds and the spatial associations among heterospecific palms can lead to different outcomes for the interacting palms species that also depend on the demographic context.

In comparing the density-dependent interactions among conspecifics, we found it surprising that the patterns of aggregations indicate that *Attalea phalerata* conspecifics experience more intense density-dependent mortality than conspecifics of *Astrocaryum murumuru*. The ability for *Astrocaryum murumuru* to persist under aggregated conditions over a larger range of cohorts compared to *Attalea phalerata* is therefore contrary to Janzen and Connell's predictions that density-dependent mortality will control the more abundant species. We hypothesize that *Astrocaryum murumuru* palms are better able to persist under aggregated conditions compared to *Attalea phalerata* because of an adaptive consequence associated with their fruiting phenologies. *Astrocaryum murumuru* palms have a distinct and synchronous fruiting period (between January and March) resulting in high densities of recruits while *Attalea phalerata* exhibit asynchronous fruiting (personal communication John Terborgh) with correspondingly lower densities of recruits. The offspring of each of the respective palm species may thus be selected to compete under different conditions of conspecific aggregation. As a result we predict that seeds of codispersed *Astrocaryum murumuru* are more likely than those of *Attalea phalerata* to recruit successfully and survive into the reproductive age under aggregated conditions.

Implications for frugivore loss. Our study highlight the potential prevalence of codispersed or contagiously dispersed species in tropical forests and the implications for

species coexistence. Although the codispersal of the two palm seeds did not ultimately leave a spatial signature on the patterns of palm adult associations, these dispersal patterns influenced the survival of palm individuals in their early life history as a result of the facilitative effects and the herd-protection to seedling and juveniles growing among heterospecific aggregates. Because palms experience different intensities of density-dependent mortality under conspecific and heterospecific aggregations, frugivores codispersing or contagiously dispersing seeds play a direct role in shaping palm species associations in the tropical forests. Incorporating these effects into models predicting frugivore loss will allow us to better predict the cascading effects of hunting on species turnover in forest communities.

Scatter-hoarding rodents, particularly agoutis, are one of the most hunted species in Neotropical rainforests. In view of hunting impacts, the loss of frugivore dispersers will likely disrupt codispersal activities of palms seeds and density-dependent interactions between *Attalea phalerata* and *Astrocaryum murumuru*. Studies have already shown that hunting impacts leads to a shift in the composition of angiosperm recruits from predominantly mammal-dispersed species to abiotically, bird or small-mammal dispersed species (Terborgh et al. 2008). In addition, we predict from our current study that hunting impacts will also select for palms producing recruits that thrive better under conspecific and heterospecific aggregations. Specifically, we predict that *Astrocaryum murumuru* palms will be more resilient than *Attalea phalerata* under situations in which hunting occurs based their ability to coexist under aggregated conditions of either conspecific or heterospecific plants. The release from competition with *Attalea phalerata* palms associated with codispersal activities may further increase the probabilities of *Astrocaryum murumuru* palms reaching reproductive age. On the other hand, we expect *Attalea phalerata* to perform more poorly under the loss of dispersal services because its

recruits are more vulnerable to density-dependent mortality under conspecific aggregation than under heterospecific aggregation. *Attalea phalerata* recruits will also suffer from the loss of herd-protection or facilitation associated with codispersal and heterospecific aggregation. Such differences in the response of palms to hunting and their resilience to frugivore disperser loss can promote shifts away from a forest with two similarly dominant palms to a forest with a single dominant palm species with unknown feedbacks for frugivores and the broader forest community.

FIGURES

Figure 2.1: *Attalea phalerata* (left) and *Astrocaryum murumuru* (right)

Figure 2.2: Palm fruits of *Attalea phalerata* (left) and *Astrocaryum murumuru* (left). Photos by Tim Paine.

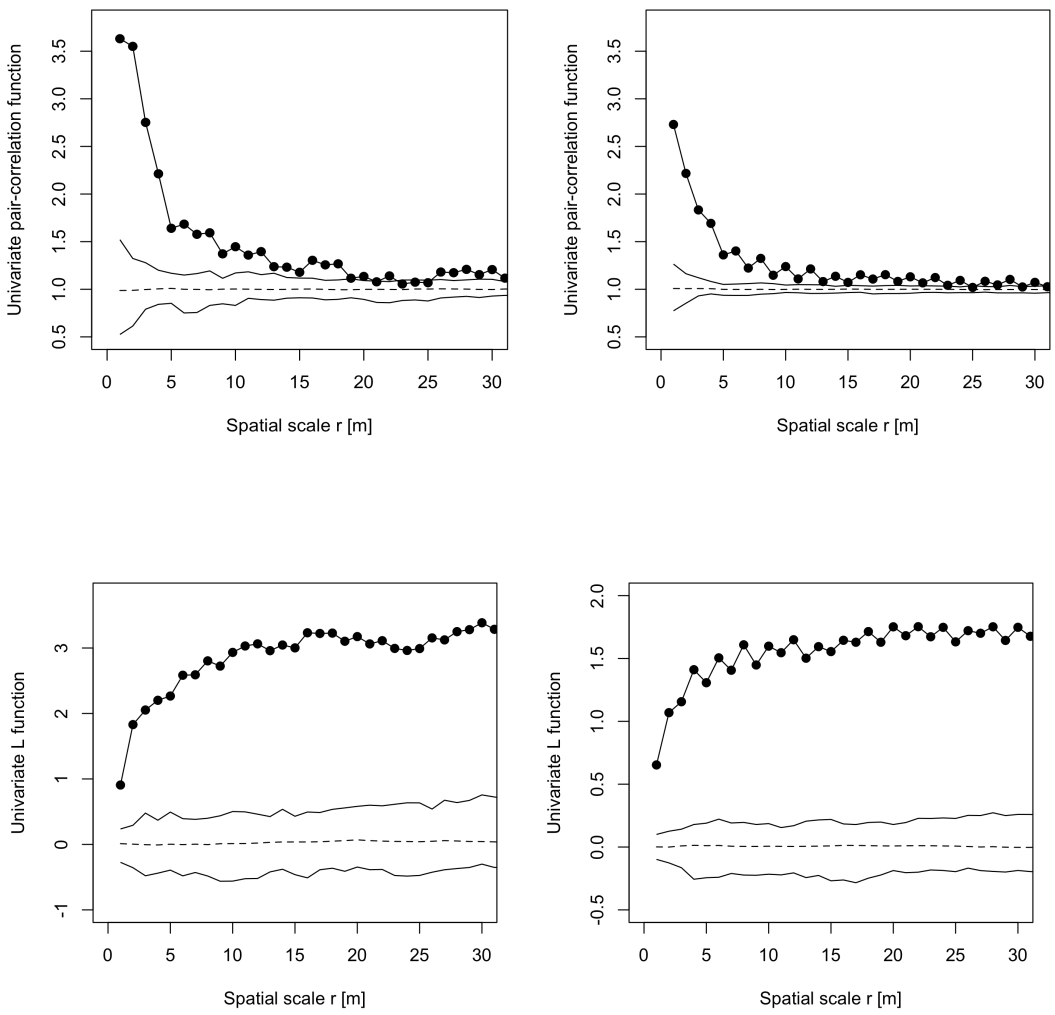

Figure 2.3: Plots of the univariate pair-correlation functions (top) and L-function (bottom) over scale *r* showing virtual aggregation and significant small-scale interactions for *Attalea phalerata* (left) and *Astrocaryum murumuru* (right). The observed data are represented as solid circles, the Monte Carlo simulation envelopes of the null model of CSR are in solid lines and the dashed horizontal line is the expected function for independent patterns.

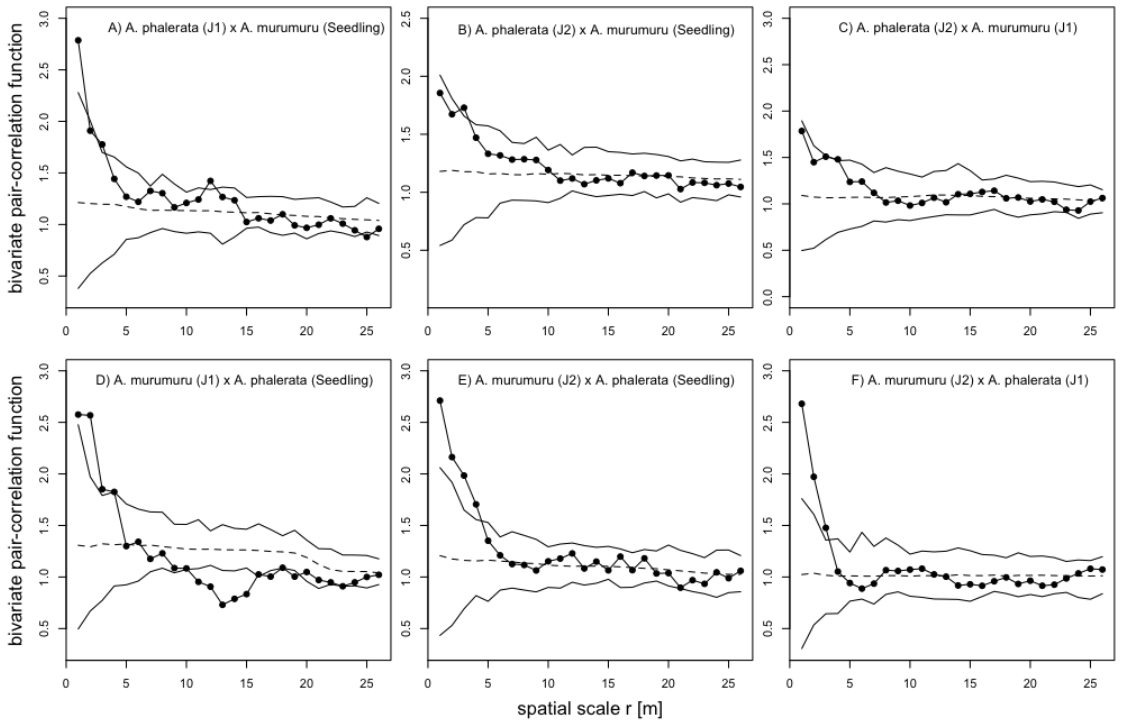

Figure 2.4: Examples of bivariate pair-correlation functions for interactions between heterospecific palm cohorts of different sizes (A-F) showing significant small-scale aggregations between 0-4m. The observed data (solid circle) is contrasted against the Monte Carlo simulation envelopes for the null model of antecedence (solid lines). The dashed horizontal line is the expected function for independent patterns

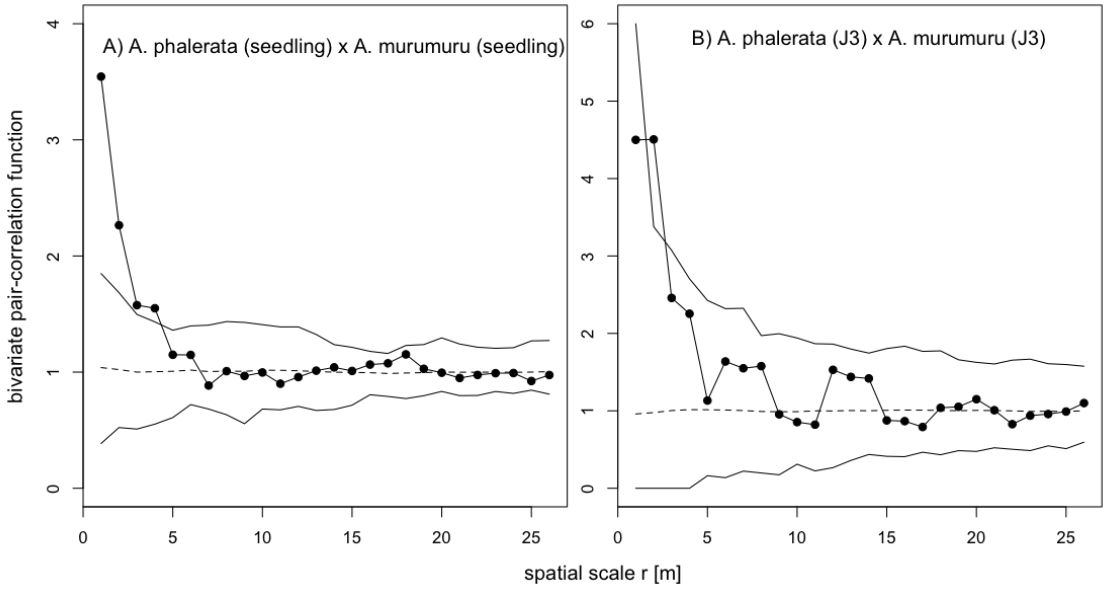

Figure 2.5: Examples of bivariate pair-correlation functions for interactions between heterospecific palms cohorts of the same size (A and B) showing significant small-scale aggregations between 0-3m and 1-2m respectively. The observed data (solid circle) is contrasted against the Monte Carlo simulation envelopes for the null model of antecedence (solid lines). The dashed horizontal line is the expected function for independent patterns

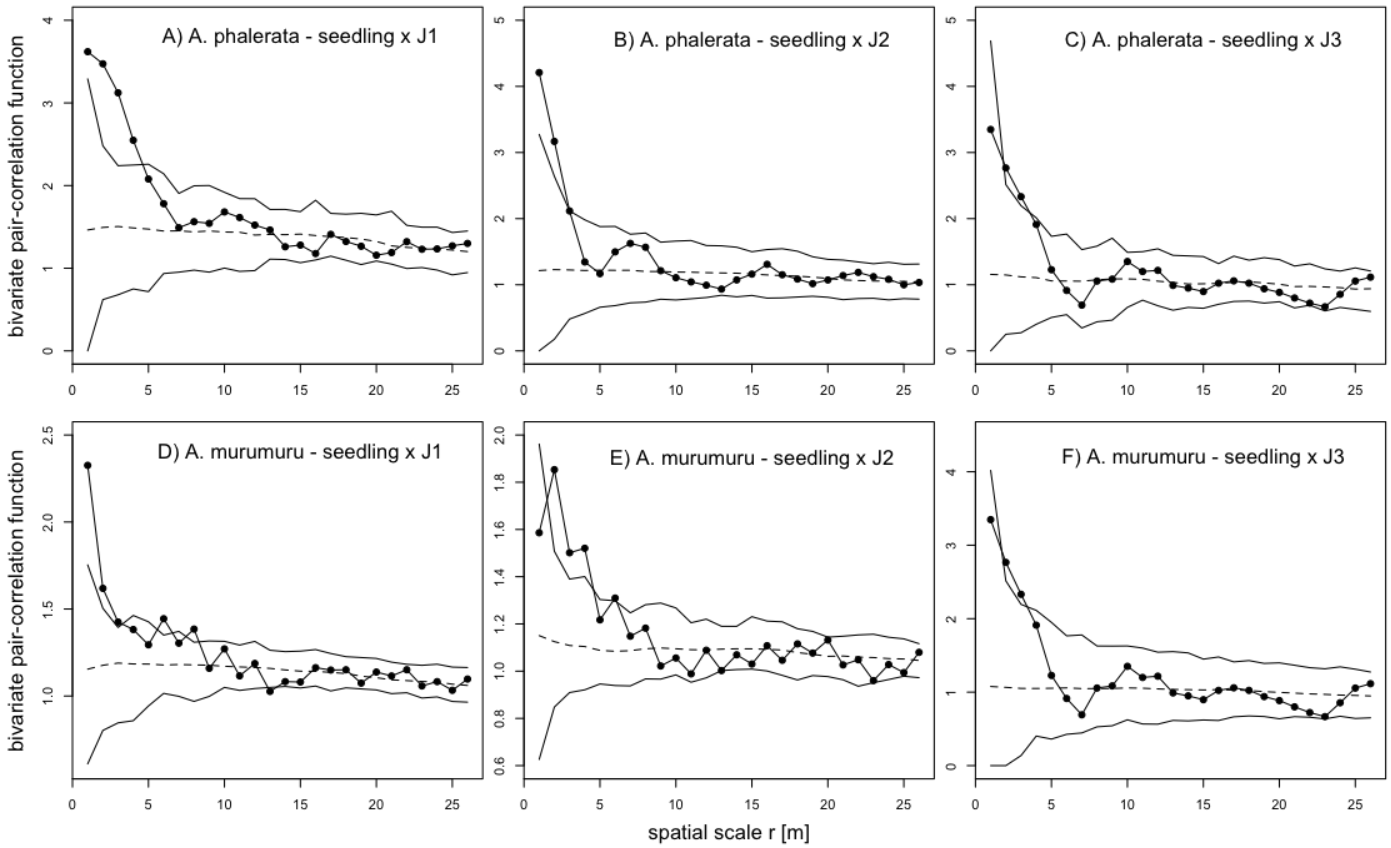

Figure 2.6: Examples of bivariate pair-correlation functions for interactions between conspecific cohorts in *Attalea phalerata* (A-C) and *Astrocaryum murumuru* (D-F) showing significant small-scale aggregations between 0-4m. The observed data (solid circle) is contrasted against the Monte Carlo simulation envelopes for the null model of antecedence (solid lines). The dashed horizontal line is the expected function for independent patterns.

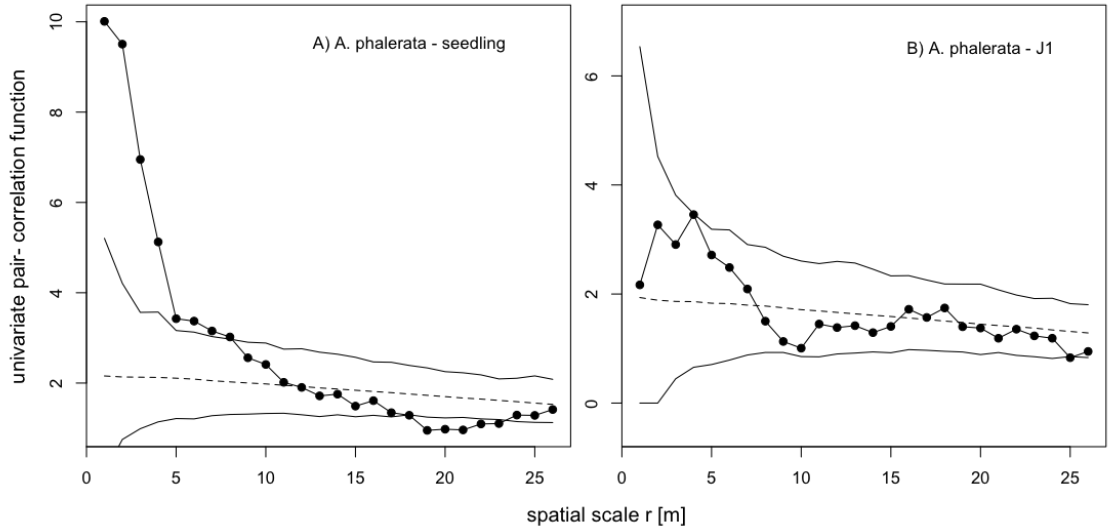

Figure 2.7: Plots of the univariate pair-correlation functions for spatial interactions among conspecific *Attalea phalerata* seedlings (A) and J1 (B) showing significant small-scale aggregation between 0-8 m and at 3 m respectively. The observed data (solid circle) is contrasted against the Monte Carlo simulation envelopes for the null model of independence (solid lines). The dashed horizontal line is the expected function for independent patterns.

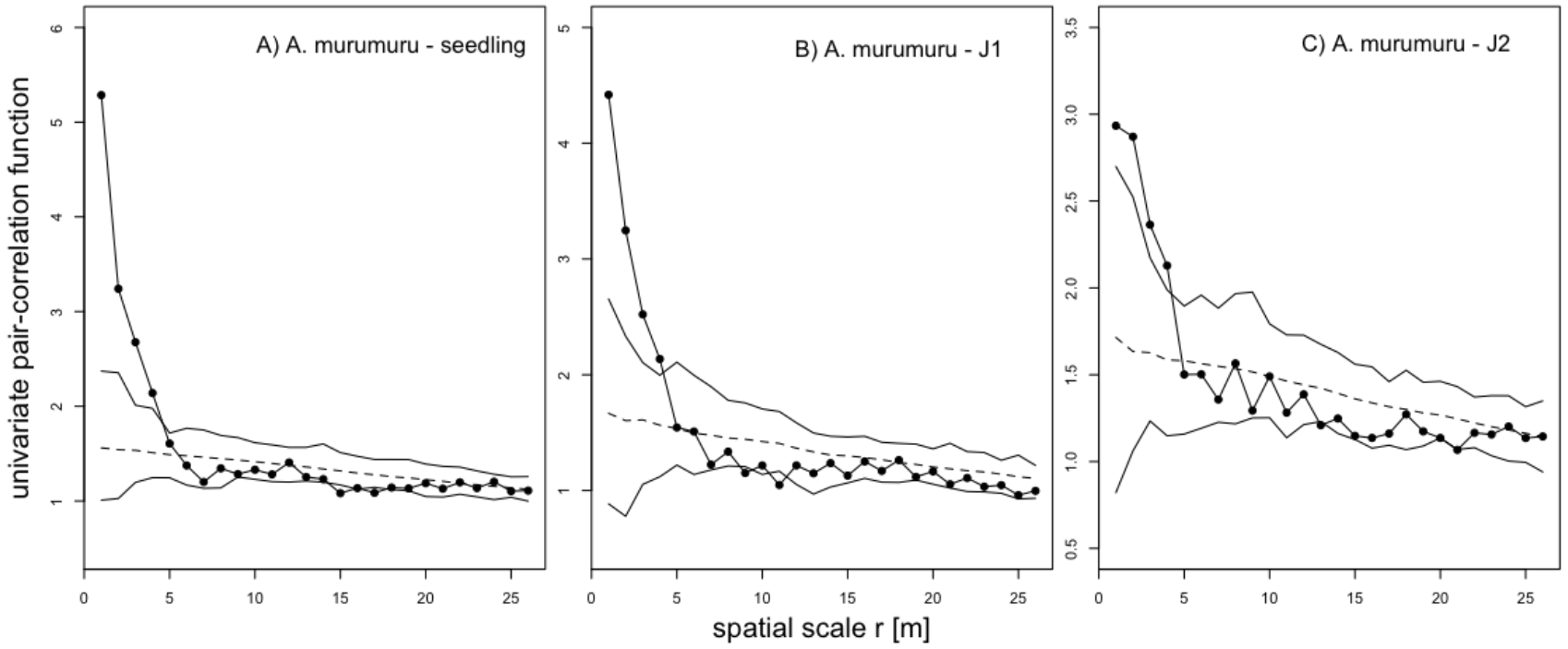

Figure 2.8: Plots of the univariate pair-correlation functions for spatial interactions among conspecific *Astrocaryum murumuru* seedlings (A), J1 (B), and J2 (C) showing significant small-scale aggregation between 0-3m. The observed data (solid circles) is contrasted against the Monte Carlo simulation envelopes of the null model for independence (solid lines). The dashed horizontal line is the expected function for independent patterns.

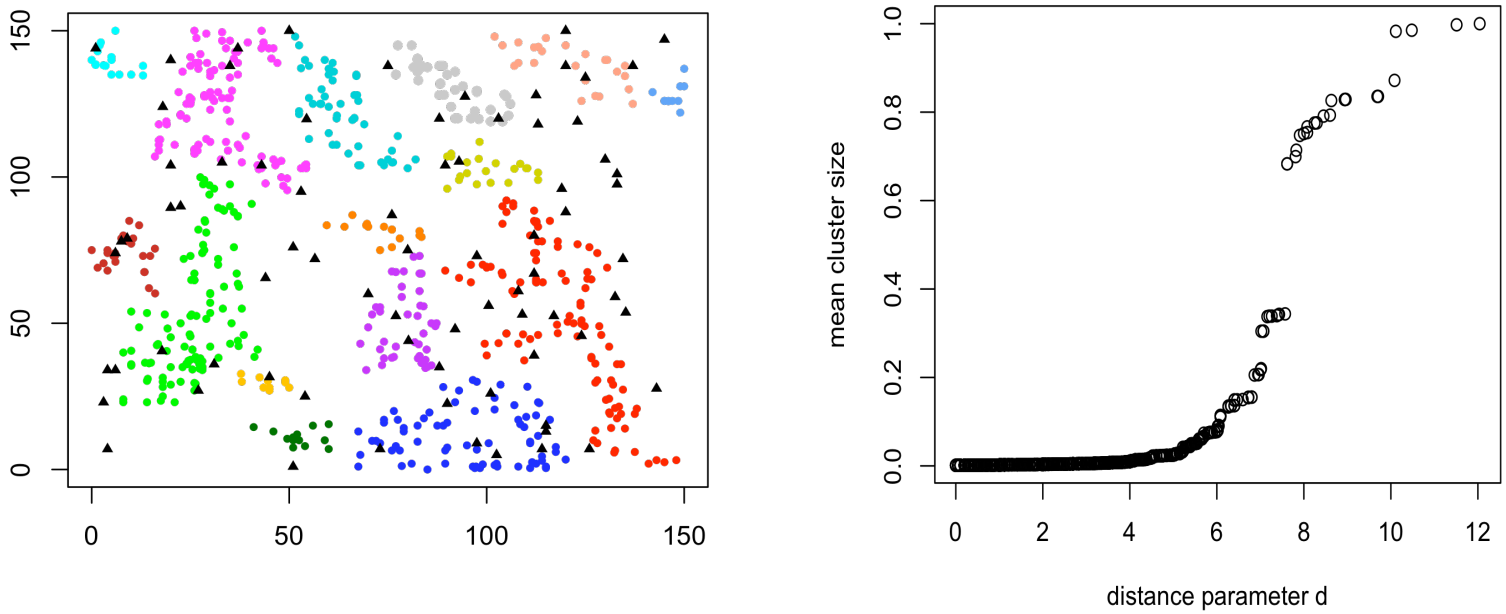

Figure 2.9: Clusters of *Attalea phalerata* and *Astrocaryum murumuru* seedlings represented by the different colors at non-random scale of aggregation (clusters of less than 10 palms are not shown here)(left). Reproductive palm adults are overlayed in black triangles. The plot on the right shows the mean cluster size of palm seedlings with a percolation threshold at a critical distance of 7.6 m.

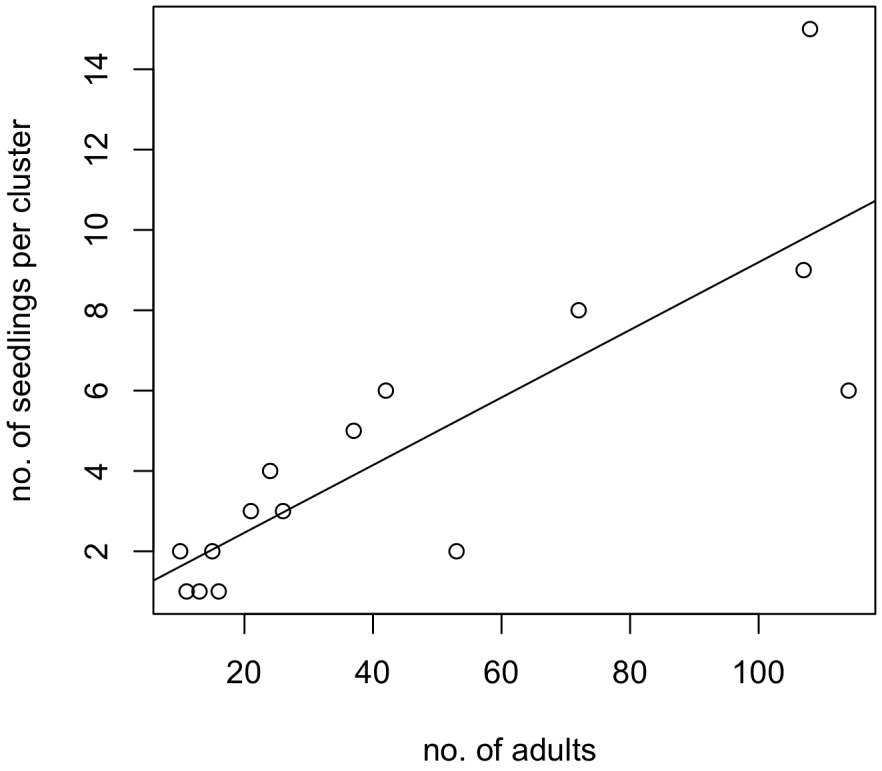

Figure 2.10: Plot of the relationship between number of seedlings per clusters and the number of reproductive adults.

TABLES

Table 2.1: A summary of the frequency and intensity of palms in each size category

	Attalea phalerata			*Astrocaryum murumuru*		
Cohorts	frequency	proportion	intensity	frequency	proportion	intensity
A	34	0.058	0.0015	43	0.027	0.0019
AJ	29	0.050	0.0013	12	0.007	0.0005
J1	136	0.232	0.0060	452	0.282	0.0201
J2	96	0.164	0.0043	454	0.258	0.0184
J3	67	0.114	0.0030	100	0.062	0.0044
B	224	0.380	0.0010	582	0.363	0.0259

Notes: A= adults, AJ= stemmed juveniles, J1=juveniles (<2m), J2=juveniles (2-4m), J3=juveniles (>6), B=seedlings

Table 2.2: Summary of interspecific spatial interactions between *Attalea phalerata* and *Astrocaryum murumuru* (row by column).

	Astrocaryum murumuru							*Attalea phalerata*					
	S	J1	J2	J3	AJ	Adults		S	J1	J2	J3	AJ	Adults
Attalea phalerata							*Astrocaryum murumuru*						
Seedlings	A	--	--	--	--	--	Seedlings	A	--	--	--	--	--
J1	A	A	--	--	--	--	J1	A	A	--	--	--	--
J2	A	A	A	--	--	--	J2	A	A	A	--	--	--
J3	A	R	N	A	--	--	J3	N	R	R	A	--	--
AJ	N	N	R	N	N	--	AJ	N	R	R	N	N	--
Adults	N	N	R	R	N	N	Adults	N	R	R	R	R	R

Notes: Spatial associations between palms of the same-sized cohorts are based on the tests of independence null model while those between different-sized cohorts are based on tests of the antecedence null model. A=aggregated, R=repulsed, N=no association. Significant departures from the null models were considered at $p<0.05$ based on 999 (for $n>300$) or 99 (for $n<300$) Monte Carlo simulations. AJ= stemmed juveniles, J1=juveniles (<2m), J2=juveniles (2-4m), J3=juveniles (>6).

Table 2.3: Summary of the intraspecific spatial interactions for *Attalea phalerata* and *Astrocaryum murumuru* (row by column).

	Attalea phalerata							*Astrocaryum murumuru*						
	S	J1	J2	J3	AJ	Adults		S	J1	J2	J3	AJ	Adult
Attalea phalerata							*Astrocaryum murumuru*						
Seedlings	A	--	--	--	--	--	Seedlings	A	--	--	--	--	--
J1	A	A	--	--	--	--	J1	A	A	--	--	--	--
J2	A	A	R	--	--	--	J2	A	A	A	--	--	--
J3	A	R	N	R	--	--	J3	A	A	A	N	--	--
AJ	R	R	N	N	N	--	AJ	A	N	R	N	N	--
Adults	A	A	N	N	N	N	Adults	A	R	A	N	N	N

Notes: Spatial associations between palms of the same-sized cohorts are based on the tests of independence null model while those between different-sized cohorts are based on tests of the antecedence null model. Interactions are indicated by: A=aggregated, R=repulsed, N=no association. Significant departures from the null models were considered at $p<0.05$ based on 999 (for $n>300$) or 99 (for $n<300$) Monte Carlo simulations. AJ= stemmed juveniles, J1=juveniles (<2m), J2=juveniles (2-4m), J3=juveniles (>6).

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Chapter 3: The dispersal and spatial population ecology of a Neotropical palm inferred by parentage analysis

Abstract: Ecological theory predicts that offspring can gain benefits from dispersing away from their parents and siblings to escape mortality associated with distance and density-dependent factors. This has been traditionally studied by examining the fate of seeds and recruits located at different distances from conspecific adults. Because it has been impossible to identify the parent-offspring and sibling relationships of individuals, it is a challenge to establish the actual spatial dynamics and interactions of dispersal, recruitment, and mortality rigorously. Molecular parentage analysis allows inference about such relationships and thus dispersal and recruitment events, opening a new dimension to the study of spatial patterns and processes of forest populations. Here we apply this technique to *Attalea phalerata,* a common palm in western Amazonia, and study the spatial distributions of adult palms and their offspring of different demographic classes. Using parentage analysis, we infer recruitment patterns and the changing spatial distribution of palm individuals over demographic classes in the context of distance and density-dependent interactions. We also investigated density-dependent effects at the seed stage, focusing specifically on the spatial intensity seed predation associated with bruchid beetles, a primary seed predator of *A. phalerata* palm seeds. The parentage, experimental, and spatial analyses yielded several findings related to the dispersal and population ecology of these palms. i) There is a high level of seed movement throughout the study area of 2.5 ha, with a high percentage of seedlings under any given adult produced by another parent; ii) there is strong evidence for density-dependent seed predation by bruchid beetles; iii) despite the high levels of movement and density-dependent predation on seeds, seedlings are significantly aggregated around parents and

with each other, but become increasingly disaggregated as they grow to maturity, consistent with density- and distance-dependent mortality*;* iv) this disaggregation effect was found for interactions between juveniles and parents, juveniles and other adults, and among juveniles; *v)* spatial genetic structure was only found to be strongest in the seedling class due to dispersal limitation and subsequent density-dependent mortality breaks up these clusters and spatial genetic associations in larger cohorts; vi) parentage analysis revealed significant reproductive skew, but interestingly the individuals that dominated reproduction varied between different cohorts implying temporal variability in reproductive success. Our findings collectively highlight the importance of seed dispersers for the life histories of palms and the maintenance of spatial population and genetic structure, and provide a foundation for understanding the consequences of loss of seed dispersing mammals on palm populations in hunted forests.

3.1 INTRODUCTION

A central question in ecology is how spatial population processes and species interactions contribute to maintaining species richness. Distance and density-dependent mortality effects are thought to be one of the key drivers of species richness in tropical forests. According to the Janzen-Connell model (Janzen 1970, Connell 1971), the spatial distribution of recruits will be determined by the interaction between two components 1) how far seeds are dispersed from parents, and 2) the probability that seeds and seedlings will survive to maturity at different distances from the parent tree or conspecific adults. The model proposes that seed-crop size and the behavior of dispersal agents are key factors influencing dispersal distance from parents, while predators of seeds and seedlings influence survival probabilities of recruits to maturity. Janzen (1970) distinguished between two types of predators – distance- and density-dependent predators. The impact of distance predators is a function of the distance between the adults and juveniles, while the influence of density-dependent predators is driven by the spatial pattern of aggregation or dispersion of individuals on a landscape. The combined activities of distance and density-dependent predators results in the mortality of recruits near parents, which opens up area for the recruitment of other species. These processes are hypothesized to promote species coexistence and maintain the high diversity tree species in tropical forests.

Because the probability of predation and mortality is thought to be inversely related to the distance from parents (i.e., seed-producing tree), seed dispersal plays an integral role in moderating the effects of predation and is a key factor in both the persistence of single species and the coexistence of multiple species. Studies have

demonstrated the benefits of dispersal for the escape from distance- and density-dependent mortality, plant recruitment success, and seedling diversity (Harms et al. 2000, Howe and Miriti 2004). The significance of dispersal to tropical forest diversity has also generated a growing number of studies investigating the implications of disperser loss resulting from the alarming rates of defaunation across the tropics (Wright et al. 2000, Terborgh et al. 2008). The Janzen-Connell model thus provides an important theoretical framework for assessing how human disturbance such as hunting of frugivore dispersers can lead to changes in population recruitment patterns and shifts in forest composition (Terborgh et al. 2008).

However there remain gaps in our knowledge of forest dispersal ecology. In particular, it has been difficult to identify the parent-offspring and sibling relationships of individuals, which are necessary to infer individual dispersal events and elucidate processes underlying spatial pattern. For example, juveniles may be aggregated around parents due to dispersal limitation, but even if dispersal is random across a plot, aggregation could form due to patches of favorable environmental conditions. Molecular methods, particularly parentage analysis, are thus useful in allowing us to develop a more integrated view of the role of species interactions and spatial processes in tree life histories.

Parentage analysis has been successfully used to untangle complex and fine-scale patterns of dispersal and recruitment patterns of individuals within a population (Hardesty et al. 2006, Jordano et al. 2007). Hyper-variable markers such as microsatellites provide a robust technique for assigning parentage and have emerged as the marker of choice for addressing questions at ecological scales because of their power to resolve population level relationships and the development of readily available computer software package for analyses (Jones and Ardren 2003).

Using microsatellite-based parentage analysis in concert with spatial analysis, we examined the dispersal and recruitment dynamics of *Attalea phalerata* Mart., a common neotropical forest palm in a pristine forest. *Attalea phalerata* is an important food resource for forest frugivores, and is both a food and material resource for human populations. Of critical interest is the relative balance of forces aggregating populations, such as dispersal limitation, and the forces disaggregating populations – dispersal, and density/distance dependent mortality and how these factors may collectively influence spatial genetic structuring within a population. Understanding the interplay between these forces and the role of animal dispersal in particular will provide a rigorous foundation to predict and assess the impact of defaunation on the persistence of palm populations.

To develop our understanding of this interplay, we examined several components of the dispersal ecology and life history of *Attalea phalerata*. First, we asked whether the youngest recruits are significantly clustered around the parent tree. Knowing the pattern of clustering will establish the strengths of dispersal limitation, which is an aggregating force, and the possible effect of density-dependent seed predation, which is a disaggregating force in seedling establishment. To evaluate the presence of density-dependent seed predation independently, we experimentally related bruchid beetle predation to palm density.

Second, by examining the spatial patterns of offspring in different cohorts from seedling to near maturity, we estimate whether surviving individual trees of an age cohort tend to become more aggregated or disaggregated as they age. We look for changing patterns in the clustering of individuals with parents, siblings, other individuals of the same cohort, and nonrelated individuals in the population at large. We also apply quantitative measures of spatial genetic structure of individuals in different cohorts, which provides an alternative measure of aggregation and disaggregation. These patterns

will help evaluate the role of density and distance dependent mortality effects within and between these different components of the population.

Third, we use parentage analysis to examine the offspring contribution of different parent individuals to different cohorts, which allows us to infer reproductive skew and how it changes over time, a key but understudied variable in plant population biology.

3.2 MATERIALS AND METHODS

Study site and species. This study was conducted in a 2.25-hectare plot (150 m x 150 m) at the Cocha Cashu Biology Field Station (CC) in Manu National Park, Peru. The forest around the CC is considered one of the most pristine in Amazonia and possesses an abundance of mammals at different sizes including capuchin monkeys (*Cebus apella*), red squirrels (*Sciurus spadiceus*), pacas (*Agouti paca*), brown agoutis (*Dasyprocta variegata*), and white-lipped peccaries (*Tayassu pecari*) that depend on the resources of the *Attalea phalerata* (Gentry and Terborgh 1990). *Attalea phalerata* are also the host to the larvae of bruchid beetles *Pachymerus cardo*, which can destroy up to 80% of seeds found under trees (unpublished data, Choo). Reproductively, *A. phalerata* is a monoecious but functionally dioecious palm. Male and female flowers on each palm do not bloom synchronously and self-fertilization is not expected among these palms (Pintaud 2008). This assurance of outcrossing allows the assignment of parentage with probabilities that are as robust as those observed among dioecious species.

Overview of methods. We genotyped and assigned the parentage (i.e., seed-producing tree) of each recruit in the palm population to parents within the study plot to infer dispersal and recruitment patterns as well as to investigate the relative influence of distance and density-dependent mortality for the population. We assumed for this study that patterns across demographic cohorts reflect the patterns affecting the palm population through time.

Palm census and tissue collection. We mapped all individuals of *Attalea phalerata* found within the 2.25-hectare plot (150 m x 150 m). For each individual encountered, we documented its size using the longest leaf as a proxy. We categorized pre-reproductive palms in the population into five distinct stages: seedlings (B; palms with undifferentiated leaves and usually < 0.5 m tall), juveniles (palms with differentiated leaves and without a trunk), which included J1 (juveniles less than 2.0 m tall), J2 (juveniles 2 to 4.0 m tall), and J3 (juveniles greater than 4 m tall), and juveniles with a distinct trunk (AJ). We collected leaf samples for all seedling and juvenile palms and from the canopy leaves of adults. All tissues samples were dried with silica-based desiccant and transported to The University of Texas at Austin for DNA extraction and genotyping.

DNA extraction. We extracted DNA from the leaf tissues using a modified CTAB protocol (Doyle and Doyle 1987). Leaf tissues (approximately 100mg) were placed in 2ml Eppendorf tubes and homogenized in 700 µl of CTAB buffer with two tungsten carbide bead for 4 minutes in a mixermill. The tubes were incubated 60-65°C water bath for 30 min and inverted 3 times during this period. We then added 700μl of chloroform–isoamyl alcohol mixture (24:1) to this mixture and inverted the emulsion 50 times. The

emulsion was centrifuged for 2 min at 12000 rpm and the supernatant was transferred to a clean tubes. An equal volume of cold isopropanol was added to the supernatant and the tubes were placed in a -20°C for 30 min. We then centrifuged the tubes for 10 min at 130000 rpm to pellet the DNA and washed the pellets twice in 500μl 70% ethanol. We centrifuged the tubes again for 5 min at 13000 rpm to resecure the DNA pellets to the tube. The DNA pellet was air-dried for 8 h and resuspended in 50 ul of TE buffer.

Parentage analysis. We used a microsatellite-based parentage analysis for our study. Fourteen microsatellites were characterized in Chapter 4 and from these we selected ten loci that did not exhibit a significant presence of null-alleles or deviate from Hardy-Weinberg equilibrium for genotyping palm recruits (i.e., seedlings and juveniles) to their parents. Details of the microsatellite library construction and development, loci screening, PCR conditions are provided in Chapter 4. We used the program CERVUS (3.03; Kalinowski et al. 2007) to reconstruct the parent-offspring relationships (parent here indicates the seed-producing tree) under a maximum likelihood framework implemented in the program. This program estimates the likelihood of maternity at both relaxed (80%) and strict (95%) confidence intervals. We selected the 80% confidence for maternal assignment, although over 82% of the offspring were assigned to a single seed-producing parent at a confidence of 95%. We used 10,000 simulation tests to calculate the critical values of likelihood. We considered offspring that were genotyped to the same parent as siblings.

Seed dispersal and movement. We inferred dispersal of recruits using the recruitment distance of offspring from adults. We also quantified the proportion of offspring versus

non-offspring recruits within a 10 m radius of each parent to assess the movement of seeds across the study plot.

Density-dependent predation. Using an experimental approach, we evaluated bruchid predation intensity across the plot and related it to variation in palm density. We monitored for a period of 14 days bruchid predation intensity on seeds and fruits across 72 locations that distributed across the study plot. We used the number of bruchid eggs found on seeds or fruits as a proxy for bruchid predation intensity. We carried out two experiments, which commenced 10 days apart due to the timing of naturally fallen *Attalea phalerata* fruits. In the first experiment, we observed predation on 6 seeds and 5 fruits (total 11) at each location and in the second experiment we recorded predation on 2 seeds and 4 fruits (total 8) at each location. We defined seeds as fruits with most of the mesocarp removed (Capuchin monkeys were responsible for this removal) and fruits were those with over 90% of their mesocarp intact.

We interpolated predation intensities and adult densities (from censused maps) across the study area using Kriging surface estimates implemented in R (Fortin et al. 2002). We used Mantel tests to compare the relationship between predation intensity and adult densities for the entire study plot. We used the Bonferroni test of global significance to account for multiple comparisons, which requires that at least one critical *p*-value is less than α/n to evaluate and reject the set of null hypotheses (Legendre 1998). In our test of significance, we used 10000 permutations following Goslee and Urban (2007). For these analyses, we used the ecodist package in R (R Development Core Team 2009). We also used generalized linear models to investigate whether adults density and predation intensities were important predictors of seedling abundance and applied the Akaike Information Criterion to select the most parsimonious model (Johnson and

Omland 2004) among the models that included either or both predation intensities and adult abundance as predictors of seedling abundance.

Recruitment patterns. We documented differences in spatial patterns of recruitment by comparing the mean nearest-neighbor distances between four pairings, 1) offspring and their parents 2) offspring and the nearest non-parent adults 3) siblings and the nearest siblings, and 4) siblings and the nearest non-sibling recruits. Following Terborgh et al. (2008) we examined the observed mean nearest neighbor-distance for each group (D_{obs}) against a null model based on the expected mean nearest neighbor distance of randomly distributed points, calculated as:

$$D_{exp} = 0.5(Density)^{-0.5}$$

If spatial associations of individuals in each group were random, D_{obs} approaches D_{exp}. However if individuals within a group were on average closer (aggregated) or further (overdispersed) to one another than the random expectation, $D_{obs} > D_{exp}$ or $D_{obs} < D_{exp}$ respectively. The distances were normalized in all cases to account for differences in densities and to facilitate comparisons between groups.

Spatial genetic structure. To investigate changes in the population spatial genetic structure across cohorts, we used the Spatial Genetic Software to calculate the spatial genetic structure and diversity of each offspring cohort (Degen et al. 2001). The dissimilarity index, Tanimoto's D, was used to examine level of genetic diversity in the loci shared among palms in the population.

$$D_{ij} = 1 - \frac{v_{ij}}{v_{ij} + y_i + y_j}$$

For this equation v_{ij} is the number of alleles that are shared by individuals *i* and *j*, y_i and y_j are the numbers of alleles are only found in individual *i* or *j*, respectively. We generated 500 permutations to test our observed data against deviations from the null model of no spatial genetic structure.

Reproductive contribution. We calculated the reproductive contribution of parents to each offspring cohort, which gives a measure of recruitment success across time. To evaluate the evennesss of the reproductive contribution, we calculated the effective number of parents (*P*) of each distribution, which is maximized when each individual is contributing equally and minimized when all recruits are derived from one parent individual. The formula for this measure is a simple transformation of the Shannon Entropy equation, $P = e^{-\sum_i p_i \ln(p_i)}$, where *i* indexes over parents and p_i is the proportion of offspring in a cohort derived from parent *i*. Specific to our study, evenness is maximized when P = 27, which means all adults in the plot are contributing equally. We also examined whether there was significant associations between the size of a parent (height and diameter at breast height-dbh) and its reproductive contribution.

3.3 RESULTS

Parentage assignment. A total of 291 palm seedlings and juveniles were genotyped to a parent within our study plot. (Fig. 3.1 and Fig. 3.2), representing 51 % of the total number of seedlings and juveniles censused. We assume the 49% of seedlings and

juveniles that were not genotyped to any of the parents within the study plot originated from seed-producing palms outside of the study plot.

Seed dispersal inferred from recruitment. The estimated dispersal distance of all offspring from parents ranged between 2.5 to 154 m with a median of 17.5 m (Fig. 3.3). These estimates indicate a high probability of non-offspring seeds reaching the seed shadow of a neighboring tree. One average, each adult tree is 14.2 m away from the nearest conspecific adult with a range of 5.9 to 34.7 m.

A significantly higher proportion of non-offspring recruits versus offspring recruits were found within a 10 m radius of parent trees ($p<0.05$; Fig. 3.4). On average, 9.7 % of the palms were offspring, while 31.7 % belonged to other parents in the plot and 57.8 % belong to parents outside of the plot. Each palm adult was spatially associated with recruits originating from approximately four different parents. This high level of population mixing and offspring transfers between adult palms affected all trees in the study plot (Fig. 3.5).

Bruchid seed predation. We found on average two bruchid eggs on each attacked seed or fruit (total 136) with the number of eggs per seed or fruit ranging between 1 and 6. Using a Mantel test, we found significantly positive spatial associations between adult densities, and predation intensities ($p<0.0001$) (Fig. 3.6). Seedling abundance in the study was positively associated with both adult densities and predation intensities according to AIC-based model selection.

Spatial patterns of recruitment. Recruitment patterns show that from the seedlings to J3 cohorts, offspring were more aggregated near adults than the random expectation

($D_{obs}>D_{exp}$). However the strength of aggregation weakened in progressively older cohorts to the AJ stage where juveniles exhibited random associations with respects to their distance from parents ($D_{obs}\sim D_{exp}$; Fig. 3.7). Similar patterns of disaggregation in older offspring were observed for the recruitment distances between offspring and the nearest non-parent adults. The overall strength of aggregation was however weaker than those between offspring and their parents. Seedling and J1 cohorts were significantly aggregated with the nearest non-parent adults than expected by random ($D_{obs}<D_{exp}$), while J2 through AJ cohorts were random ($D_{obs}\sim D_{exp}$) or overdispersed ($D_{obs}>D_{exp}$) with respects these adults.

Distance between recruits indicated the strongest association was between siblings, while recruitment patterns between offspring and non-offspring recruits or those among all recruits were close to random. Specifically, distances from the nearest siblings, offspring showed disaggregation from the youngest to the oldest cohort. Seedlings to J3 cohorts were more aggregated than random to the nearest sibling in the same cohort ($D_{obs}<D_{exp}$), while AJ cohorts were close to random with respect to their nearest sibling. The spatial associations of offspring and the nearest non-sibling recruit were slightly overdispersed for the seedling and J1 cohorts, slightly aggregated for J3, and was random for AJ ($D_{obs}=D_{exp}$). Finally, the distance among all offspring and the nearest conspecific recruit (regardless of relationship) showed weak aggregation in the B, J1, and J3 cohorts, and random patterns for the J2 and AJ cohorts.

Spatial genetic structure. Seedlings and J1 cohorts exhibited the strongest genetic structure. Patch diameter of genetically similar individuals ranged up to 40 meters. Juveniles in the J2 and J3 exhibited weaker spatial genetic structure of less than 20 m. Samples sizes in AJ cohorts were too low for spatial genetic structure analysis.

The diversity of parental genes in the population of recruits on the other hand decreased in older offspring cohorts. Seedlings were the most diverse group consisting of parental genes from all parents in the study plot, while J3 cohorts were the least diverse with fewer than half parents the parents represented (Fig. 3.10).

Reproductive contribution. The overall reproductive contributions among parent palms varied widely with a mean of 10.59 $\pm$ 11.16 (mean $\pm$ standard deviation). Nearly 51% percent of genotyped offspring were produced by five parent palms in the study plot (Fig. 3.8). There was also no significant association between the dbh or height of parents and their total reproductive contribution (dbh: $F_{1,24}$=0.1019, p-value=0.75; height: $F_{1,24}$=0.64 p-value=0.31)

In terms of the evenness in reproductive contribution of parents, we found across cohorts, there is a skew in the contribution of parents to the offspring population. The effective number of parents range between 10 and 15 (Fig. 3.9). This represents between 30-50 % of the population of reproducing adults in the plot.

3.4 DISCUSSION

Our study begin to form an integrated picture of the dispersal and population ecology of *Attalea phalerata*. Parentage analysis allowed the inference of individual dispersal events, which provides a powerful window into the dynamics of dispersal and recruitment. We found that there was a striking level of seed movement across our plot due to frugivore dispersers, with over 30% of recruits within 10 m of a given adult produced by other parents in the study plot. Likewise, over 50% of recruits in the study

plot originated from parents outside the study plot. Although such high frequencies of seed transfer to habitats near other trees been documented for bird-dispersed trees (Hardesty et al. 2006, Wang et al. 2007, Sezen et al. 2009), this is the first record for a primarily mammal dispersed species. These findings underscore the importance frugivore dispersers in contributing to plant-plant interactions between "unrelated" (non-sibling recruits or non-parent adults) individuals in the population.

Despite these high rates of movement and strong experimental evidence for density-dependent seed predation by bruchid beetles, there remains a clear statistical signature of dispersal limitation in the spatial associations of offspring and parents. These results suggest that seed near parents are successfully escaping predation. We suggest two possible scenarios that may promote this outcome. First, under high resource abundance, predator satiation can result and facilitate the escape from predation. This phenomenon was previously suggested by Janzen (1970) and also been documented in other tropical plant systems (Augspurger and Kitajima 1992). Second, high resource abundance can promote the caching activities of frugivores near parents as rodent seek to minimize the energetic cost associated with hiding seeds from other competitors (Moore et al. 2007). Additional experiments will be necessary to clarify the relevance of these mechanisms to seeds escaping predation and contributing to aggregated patterns observed between offspring and patterns. Of interest is also whether seed dispersed near non-parent adults experience escape mechanisms similar to those found between parents and offspring. These findings highlights how interactions between the behaviors of predators and dispersers may influence escape patterns and the availability of suitable recruitment sites.

Parentage analysis also allowed us to address the complex relationship between dispersal and escape from the distance and density-dependent mortality. Our spatial

analysis indicated that recruits were also aggregated with non-parent adult in the study plot. This indicates that palms dispersed away from parents but recruited near non-parent adults may not benefit from the escape of distance or density-dependent predation and mortality and may indeed suffer from equally high probability of mortality as offspring near parents assuming similar negative distance density-dependent factors are involved (see discussion below). Survival probability of recruits therefore does not correspond only with dispersal but may depend more specifically on the interaction between distance and the location where seeds are dispersed. This issue was previously highlighted for bird-dispersed plants. Because avian dispersers frequently defecate the seeds of one parent under another as they move between conspecific trees to feed or rest (Clark et al. 2004), clumps of seeds and seedlings recruits from other parents are frequently found under or near other conspecific trees and it was suggested that density dependence among these dispersed recruits would be high.

The results from our study now indicate that mammal frugivore dispersers can also be responsible for promoting offspring aggregation near non-parent trees. Frugivore dispersers such as agoutis routinely cache seeds near trees (Smythe 1978). Thus, as agoutis disperse palm seeds away from a seed-producing tree, they may also preferentially cache these seeds near other adult palms. These dispersed seeds may therefore suffer from similar distance and density mortality experienced by their siblings found near parents. The idiosyncratic seed dispersal and caching behaviors of frugivores suggest that each frugivore species may contribute differentially to the survival of palm recruits depending on how far they move seeds as well as the specific microhabitats where they deposit them. These findings have implications for understanding the impacts of hunting on frugivore because it indicates that hunting impacts can only be accurately

assessed by partitioning the relative contribution of each frugivore species to palm recruitment success.

The progressive loss of aggregation between offspring and parents, offspring and non-parent adults, and siblings in larger cohorts provides strong support for distance and density-dependent mortality. There was no evidence that offspring had stronger or weaker repulsive effect with parents than other non-parent adults. This suggests common mechanisms, such as competition or pathogen spread, may be involved in disassociating offspring from palm adults over time. Because we did not come across palms that were gravely affected by herbivore damage in our census, we suspect the mortality of offspring near parents may be driven by interactions with the accumulation of below-ground organisms near parents rather than those above-ground. Studies show that soil pathogens associated with the roots systems of parent trees can significantly decrease the survival of offspring near parent trees (Packer and Clay 2000). Further investigations into the influence of soil fungal pathogens near palm parents may provide clues to the mechanisms driving the increased offspring survival away from parents. Our results also do not support Janzen's (1970) hypothesis that distance or density-dependent predators acts primarily on the seed and seedling stage to generate the overdispersion of conspecific individuals facilitating the recruitment of other species

The outcome of distance and density-dependence mortality was also reflected in the spatial genetic structure for the different offspring cohorts. Spatial genetic structure was strongest and genetic diversity was highest at the seedling stages. However the onset of distance and density-mortality among siblings resulted in a lower spatial genetic structure and reduced genetic diversity in older cohorts. Similar decreases in the spatial genetic structure of larger tree cohorts have observed in another tropical tree *Simarouba amaraba*, an animal-dispersed species, and post-dispersal mortality was suggested as one

of the contributing factors (Hardesty et al. 2005). These findings indicate that distance and density-dependent mortality not only influences the available habitats for recruitment of other species as Janzen originally proposed, it also has intrinsic impacts on the populations through genetic diversity and structure.

Parentage analysis also provided a unique insight into the relative reproductive contribution of parents to the recruit population. We found differential contribution of parents across the different offspring cohorts in our study and also the lack of any particular palm tree dominating reproductive contribution over time. Several factors involving pre- and post-seed dispersal phase may interact to contribute to these patterns. In the pre-dispersal phase, factors such as variation in seed production over time can influence parental contribution to the recruiting population (Herrera et al. 1998, Nathan and Muller-Landau 2000). Seed production is in turn affected by intrinsic factors such as the size of plant, or by extrinsic factors such as the availability of pollinators and nutrients (Hubbell 1980, Steffan-Dewenter et al. 2001). Our results however showed no correlation among the size of parents and their reproductive contribution, which suggests either size does not play a significant role for seed production, or more probably, differential survival and mortality of offspring in the post-dispersal phase blurs the relationship be parent size and reproductive contribution. As we have shown previously offspring survival is intimately tied to the activities of frugivores and how they mediate distance and density-dependent mortality. As a consequence, the reproductive output of parents and consequent survival of offspring may not covary.

In addition to the influence of dispersers, the timing of fruit production of *Attalea phalerata* palms may also influence the survival of recruits and therefore parental contribution to the recruit population over different years. Because *A. phalerata* palms in our study site fruit asynchronously (*pers. obs.; pers. comm.* John Terborgh), the recruits

for each parent palm may face distinct environmental conditions or resource availability specific to the period of each seedfall. Further studies combining parentage analysis and long-term field observations to evaluate the consistency of inter-annual fruiting periods for individual parents will be necessary to determine how variation in climatic or local environmental conditions influence seed production and the survival probabilities for recruits in different years. Overall it is evident that a combination of factors may be responsible for variation in reproductive contribution of each parent over time and each of these can have a direct influence on the genetic diversity of palms reaching reproductive maturity and the genetic composition of future palm generations.

Genetic diversity and evenness in parental contribution indicate a high attrition in the contribution of parental genes to the recruiting population. Indeed, we found a nearly two-fold decrease the genetic diversity of juveniles reaching reproductive age. Assuming these results are consistent through time, it would be interesting to examine if high levels of attrition in the parental genes to the next generation combined with introduction of parental genes originating form outside of the study plot will lead to high turnover in the genetic composition of future recruits in this palm population. This area of research is under-investigated and will benefit from investigating the influence of hunting, habitat fragmentation, and logging of palm trees for weevil cultivation, on population genetic composition, turnover, and bi-parental inbreeding.

In summary, our study has demonstrated how integrating field, spatial, and molecular investigations can allow us to address and untangle some of the complex interactions between dispersal, distance and density-dependent mortality on the recruitment patterns and spatial genetic structure of *Attalea phalerata* palms. More significantly, these approaches and the findings of our study will provide the foundations

for investigating the impacts over-hunting and forest disturbance across the tropics and their implications for the future of palm populations.

FIGURES

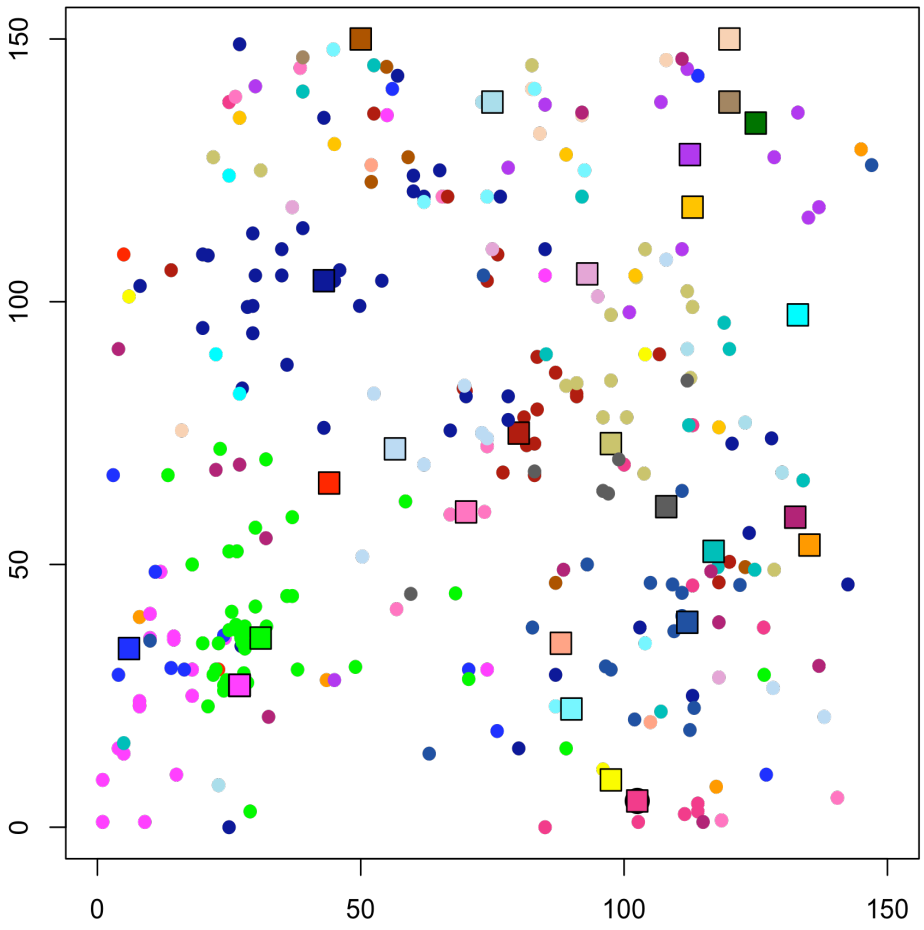

Figure 3.1: Plot of the parents (squares) and genotyped offspring (circles). Parent and offspring groups are plotted in the same colors.

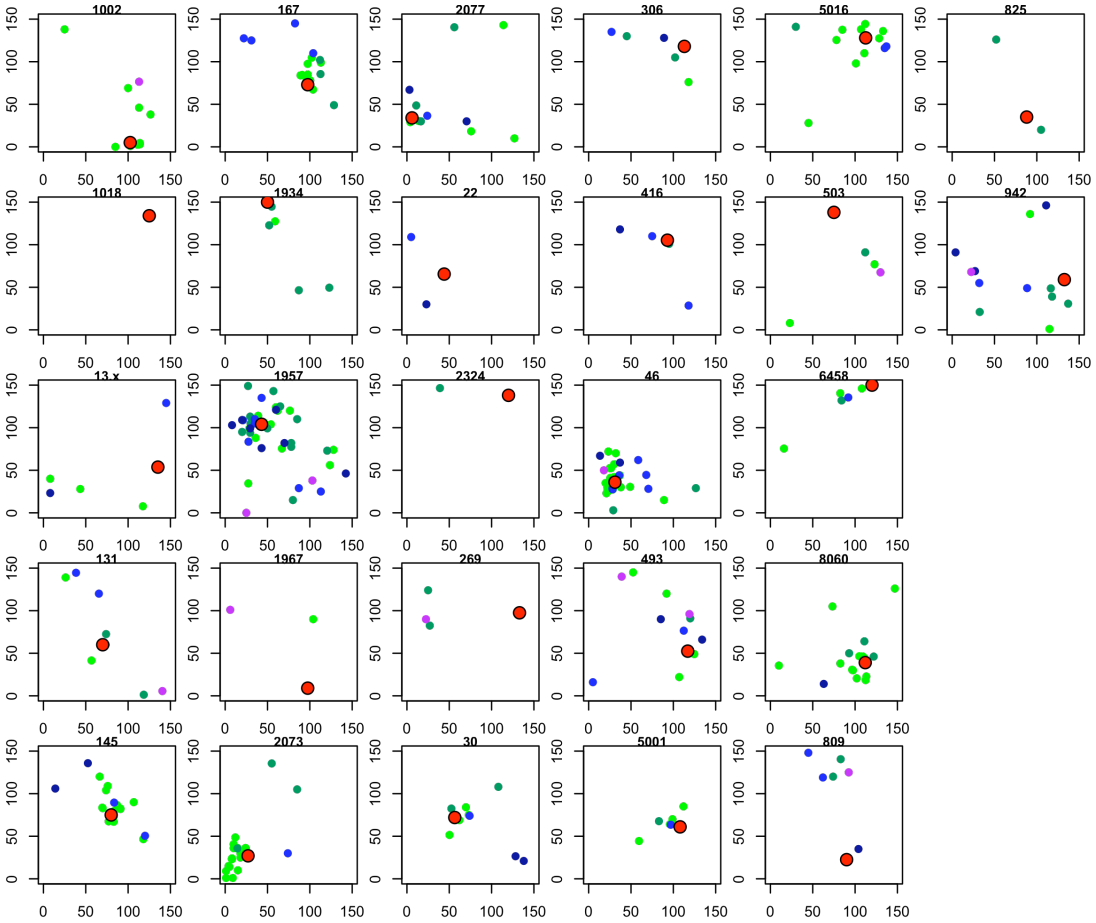

Figure 3.2: Plot of the locations of individual parent and their offspring. The numbers above each plot represents the unique identification of each parent. Parent 46, 145, 1957, 2073, and 8060 collectively contribute to 51% of the total number of offspring genotyped to parents in the study plot. Red circles are parents, green circles are seedlings, dark green circles are juveniles <=2.0m (J1), blue circles are juveniles<=4.0m (J2), dark blue circles are juveniles>4.0 m, and purple circles are juveniles with trunk (AJ)

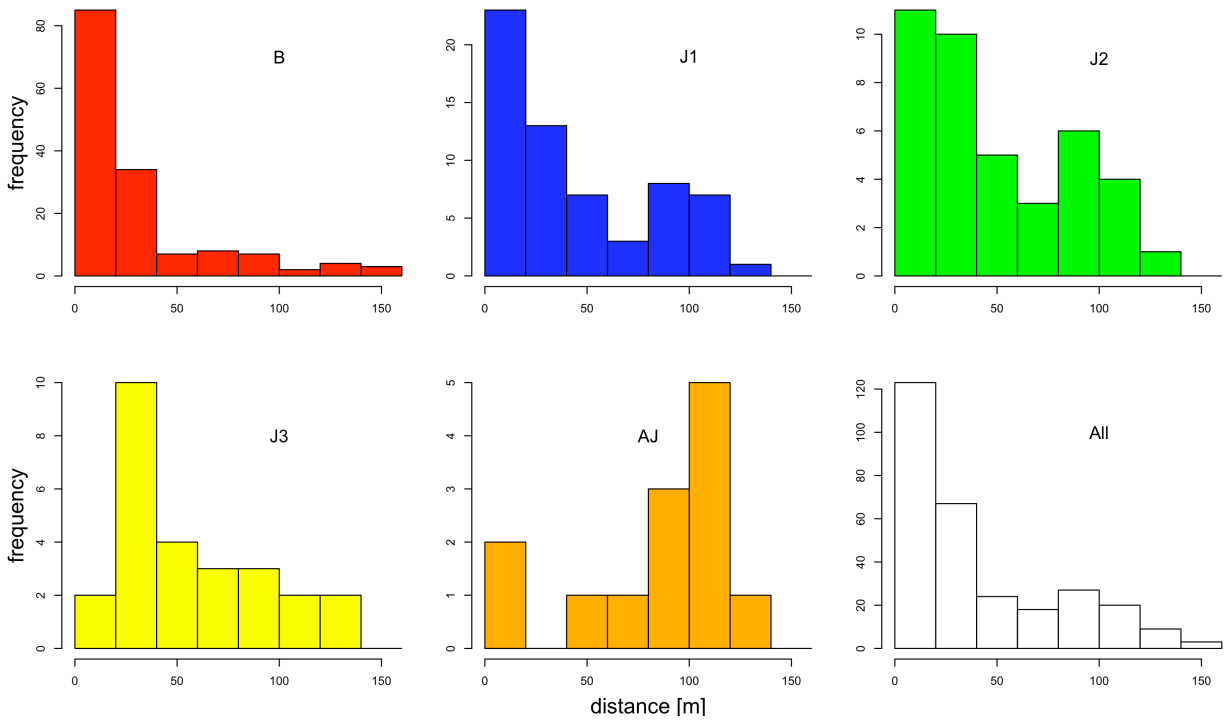

Figure 3.3: Histogram of the frequency distribution of dispersal/recruitment distances from parents for all recruits combined and for each offspring cohort (B: seedling, J1: juveniles <=2.0m, J2: juveniles<=4.0m, J3: juveniles>4m, and AJ: juvenile with trunk). The histogram break between bars is 20 m. Mean distance from parent increases with life stage.

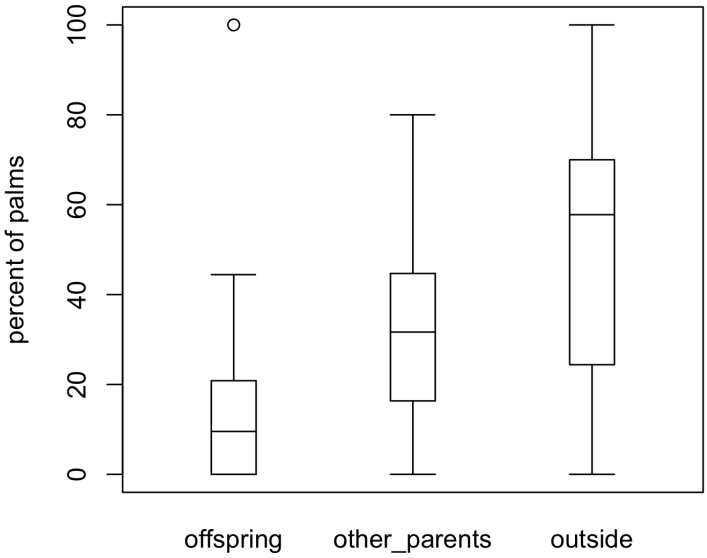

Figure 3.4: Boxplot of the relatedness of recruits found within a 10 m radius of a parent. Significantly lower proportions of offspring recruits were found near the parent compared to the proportion of non-offspring recruits that belonged to other parents in the study plot or to parents outside of the study plot ($p<0.05$).

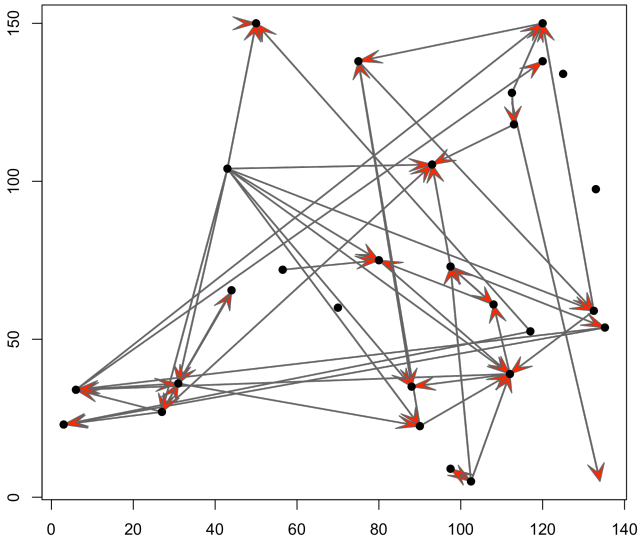

Figure 3.5: Plot showing the direction of offspring movement to habitats near other parents. The arrows indicate the direction of offspring transfer from parent to the vicinity (10 m radius) of another adult tree in the study plot. The black dots represent the adult trees. Trees without arrows were associated with recruits dispersing from parents outside of the study plot

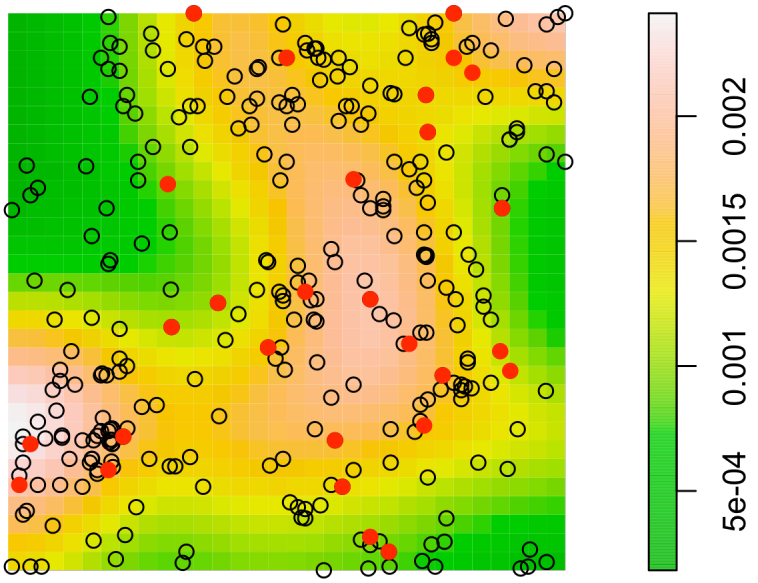

Figure 3.6: Density map of the interpolated bruchid predation intensity on palm seeds and fruits. The color range from green to beige represents low to high predation intensity. Seedling and (black circles) and adult (red circles) palm locations were superimposed onto the map.

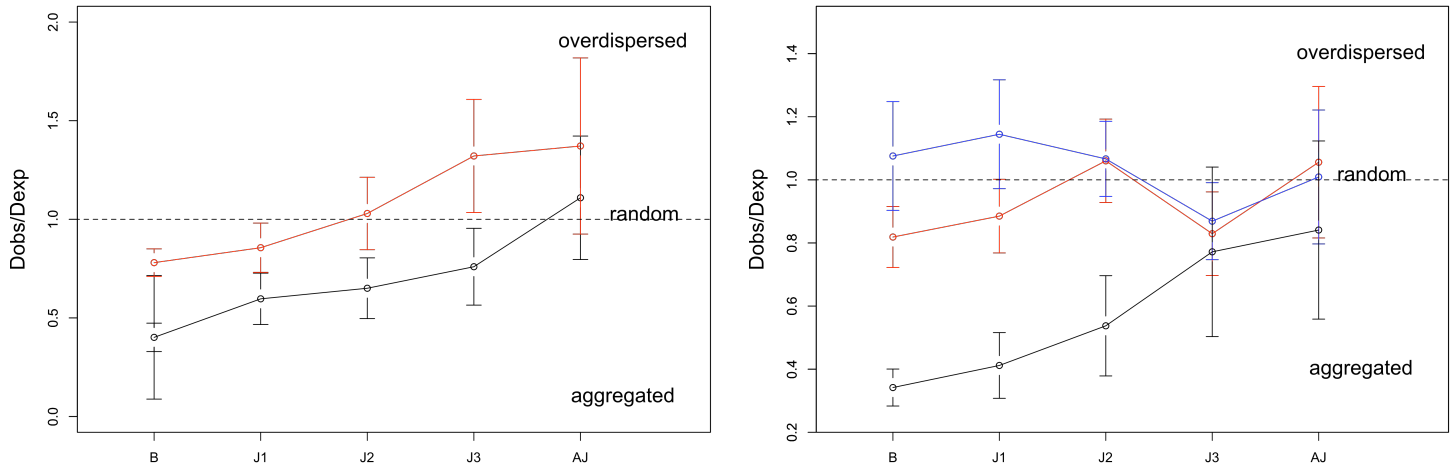

Figure 3.7: The line plot on the left shows the mean normalized distances of each offspring cohort from parents (black) and from the nearest non-parent adult other than the parent (red). The line plot on the right shows the mean normalized nearest neighbor distances between siblings (black), between sibling and the nearest non-sibling recruit (blue), and between all recruits (red). The dotted lines in both plots represent the expected mean normalized recruitment distance for random points. Points above the line indicate overdispersion while points under the line indicate aggregation.

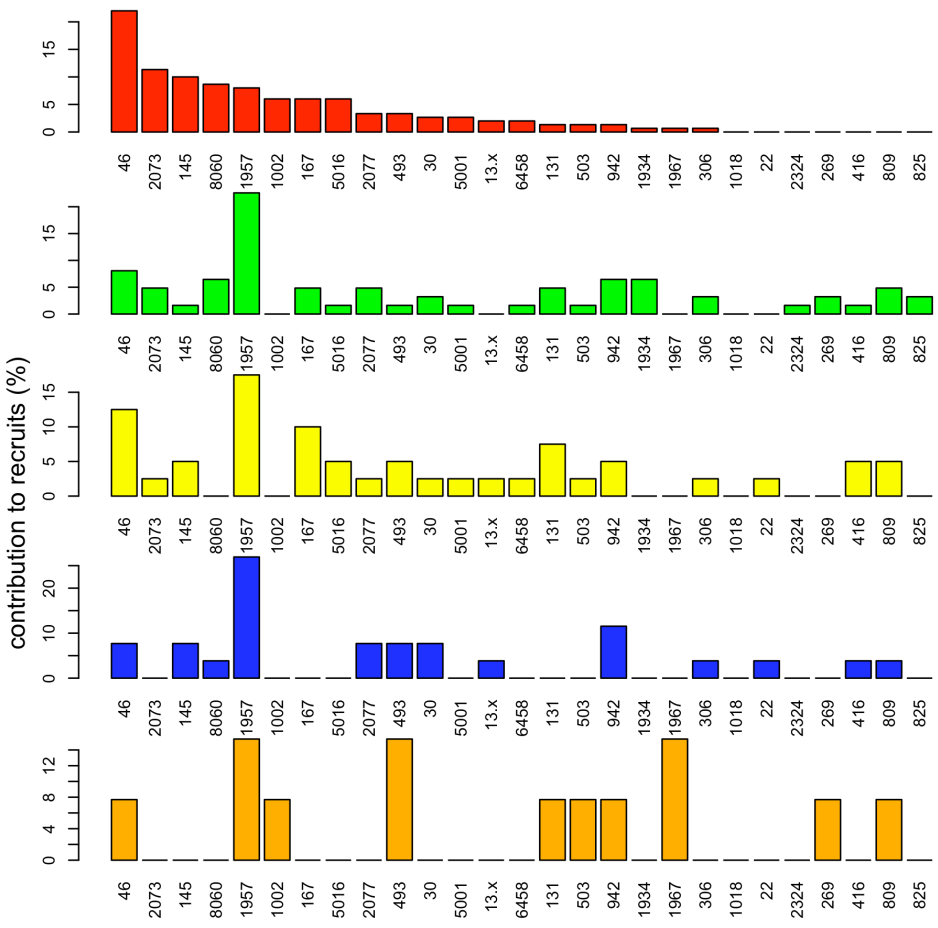

Figure 3.8: Barplot showing the individual contribution of parents to each cohort. Numbers on the x-axis indicate the individual identity numbers of each parent. Barplots in red, green, yellow, blue, orange represent B, J1, J2, J3, and AJ cohorts respectively

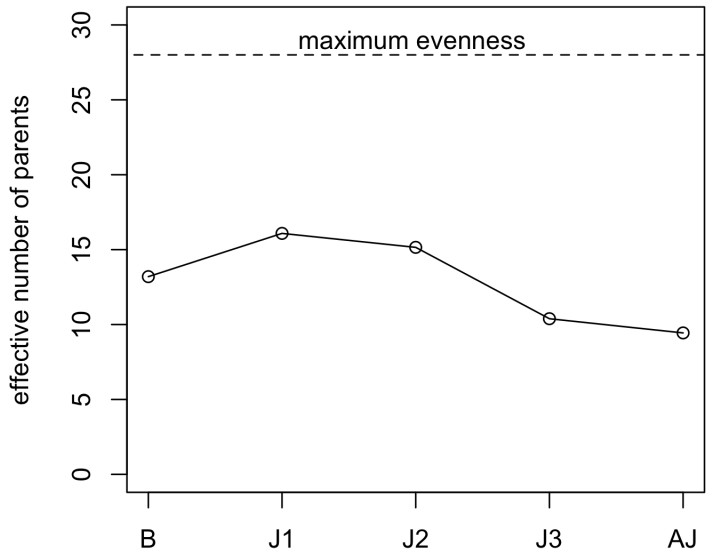

Figure 3.9: Plot of the effective number of parents, a measure of the evenness of reproduction, based on the Shannon entropy index for each cohort (see methods).

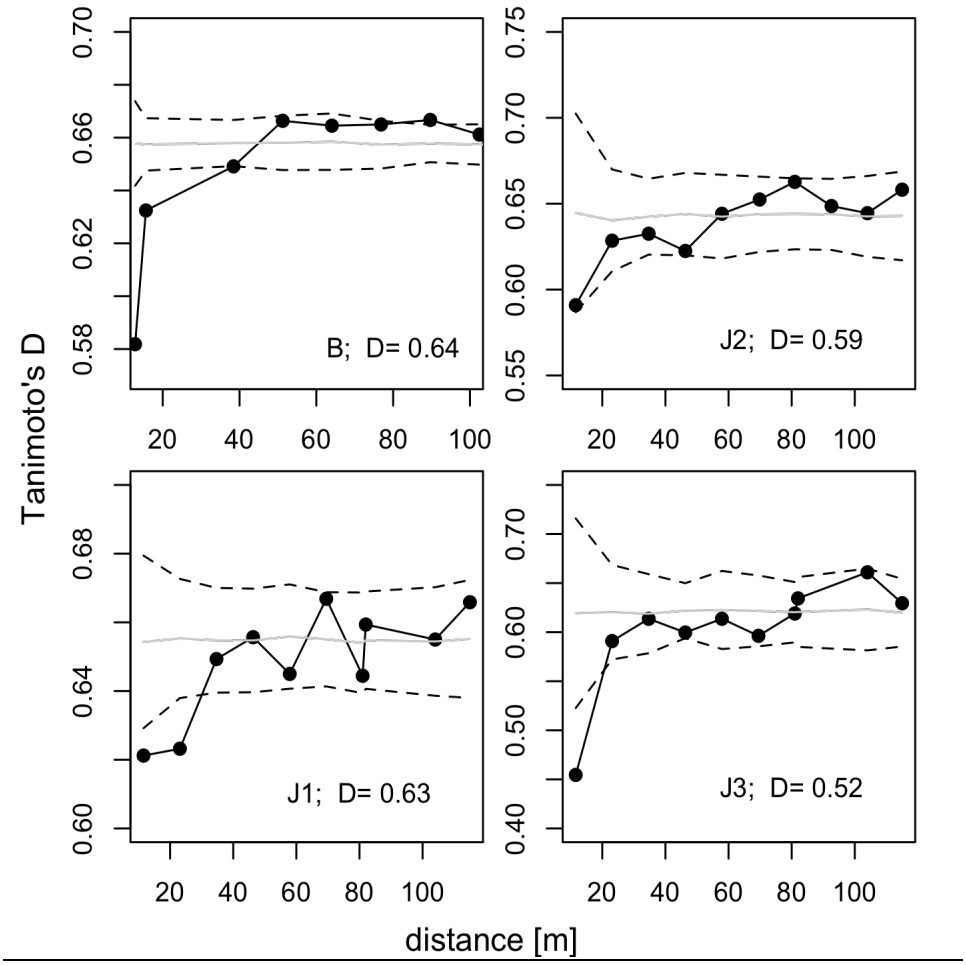

Figure 3.10: Spatial genetic structure of seedlings (B) and juveniles (J1 to J3). The line-connected points show the observed data, the dashed lines are the 95% confidence intervals, and the grey lines show the expectation for random genetic distribution. The Tanimoto's diversity index is lowest in the largest cohorts, but the spatial genetic structure is strongest in youngest cohort.

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Chapter 4: Characterization of 14 microsatellite loci in a tropical palm *Attalea phalerata*

We developed microsatellite primers for the widely distributed tropical palm *Attalea phalerata* for studies on the dispersal and spatial genetic structure of palm populations. Fourteen di-, tri-, and tetra-nucleotide microsatellite primers pairs were identified. The number of alleles in the population tested ranged between 3 and 25, with a mean of 12.1. Ten microsatellite loci exhibited no significant deviations from Hardy-Weinberg Equilibrium or presence of null alleles and their combined probability of exclusion was 0.998. These microsatellite loci will be useful in parentage analysis and population genetics studies of *Attalea phalerata*.

4.1 INTRODUCTION

Attalea phalerata is widely distributed along the periphery of the south and western Amazonia (Henderson et al. 1995). It is a monoecious but functionally dioecious palm; male and female flowers on each palm flowers asynchronously, which prevents self-fertilization (Pintaud 2008). *Attalea phalerata* is also ecologically and culturally important. A diversity of frugivores, including scatter-hoarding rodents (e.g., *Agouti paca*, *Dasyprocta punctata*, *Sciurus* spp.), primates (e.g., *Cebus apella*), and tapirs (*Tapirus terrestris*) depend on the fruit resources of *A. phalerata* and they also disperse the seeds of this palm (Quiroga-Castro and Roldán 2001, Choo 2010). Although palms in

the genus *Attalea* produce seeds with stony endocarps, undispersed seeds or those found near maternal trees suffer high rates of mortality from the predatory activities of bruchid beetles (Wilson and Janzen 1972, Wright et al. 2000). The loss of frugivore dispersers through anthropogenic activities, especially hunting, will likely have significant impacts on the dispersal, recruitment success, and population genetics for *A. phalerata*. Here, we characterized 14 polymorphic loci for *A. phalerata* that will be useful for determining the parentage of dispersed seeds and seedlings and understanding the genetic impacts of hunting on the dispersal and population genetic structure of palm populations.

4.2 METHODS AND RESULTS

To construct the genomic library, leaf tissues was obtained from a single *Attalea phalerata* palm individual collected within a 2.25-hectare study plot established at the Cocha Cashu Field Station, Madre de Dios, Peru (11.8S, 71.4W). DNA from the leaf tissues was extracted using the DNeasy plant tissue kit (QIAGEN). The microsatellite libraries were developed by Genetic Identification Services following the methods of Jones et al. (2002) and from approximately 100ng of genomic DNA. The libraries were enriched for four repeat motifs -- $(GA)_n$, $(CA)_n$, $(AAC)_n$, and $(TAGA)_n$. One hundred clones were sequenced and primer pairs were designed for 75 unique sequences using DesignerPCR version 1.03 (Research Genetics Inc.). We added a HEX labeled M13 tail to each forward primer (5'-TGT AAA ACG ACG GCC AGT-3') following Schuelke (2000).

We used 36 individual *Attalea phalerata* palms, which included 32 reproductive adults, to test the amplification and polymorphism of each locus. DNA from these individuals was extracted from silica-dried leaf tissues using a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Doyle and Doyle 1987). PCR was conducted for individual pairs of primers in 10 μl reactions volumes containing 20ng/ul of template DNA, 1x PCR buffer, 2.5 mM MgCl, 1 mg/ml BSA, 0.03 μM forward primer, 0.25 μM reverse primer, 0.4 μM forward dye-labeled M13 primer, 0.2 mM dNTPs and 1U Taq polymerase. The PCR conditions were 94°C for 2 min, 30 cycles at 94°C for 45 s, 55°C for 45 s, 72°C for 30s, and a final extension of 72°C for 10 min. We ran PCR products on a 2% agarose gel stained with SYBR safe (Invitrogen) and used UV light to visualize them.

Fourteen loci showed reliable amplification and allelic polymorphisms that we could score reliably (Table 1). For these 14 loci, we replaced the general M13 forward with individually labeled fluorescent primers and grouped them into three multiplex PCR reactions namely P, Q, and R. Multiplex P comprised of primer B121_4, C11_7, D106_3, and D124_5; multiplex Q included A106_2, C5_4, D2_1, D3_2, and D110_8; and multiplex R composed of A103_3, B101_4, B102_8, B103_3, and C122_3. The multiplex PCR conditions were: 94°C for 3 min, 30 cycles each of 94°C for 30 s, 52°C (multiplex P)/ 63°C (multiplex Q)/ 60°C (multiplex R) for 45 s, and 72°C for 45 s, and a final extension of 72°C for 10 min. PCR products were analyzed on an ABI 3100 Genetic Analyzer and alleles were scored using SoftGenetics GeneMarker v1.7 (State College,

PA). For each locus, we tested for presence of null-alleles and deviations from Hardy-Weinberg Equilibrium (CERVUS; Kalinowski et al. 2007).

We tested the multiplexed primers on 569 *Attalea phalerata* individuals in a population of palms in a 2.25-hectare plot experimental plot at Cocha Cashu. All 14 loci were polymorphic with the numbers of alleles per locus ranging between 3 and 25 and the average number of alleles was 12.1 (Table 1). The observed heterozygosity ranged between 0.26 and 0.89 with a mean of 0.68. Four loci D2, D3, D110, and C122 showed significant departures from Hardy-Weinberg equilibrium and null-allele frequencies greater than 0.05 (Table 1). These four loci should therefore only be included for parentage analysis with programs that can accommodate bias estimates associated with null alleles (Pemberton et al. 1995, van Oosterhout et al. 2004). The remaining 10 microsatellite loci have a combined probability of exclusion of 0.998 for the first parent and thus provide considerable power for parentage studies (CERVUS; Kalinowski et al. 2007).

4.3 CONCLUSION

Of the 14 microsatellite markers developed for *Attalea phalerata* parentage, 10 were found to be reliable and sufficiently polymorphic for parentage analysis of *Attalea phalerata* individuals within a population. These markers will be useful for local scale studies aimed at inferring the relative contribution of seed dispersal and/or pollination to gene flow and population genetic diversity.

Table 1: Characteristics of 14 polymorphic microsatellite loci amplified in *Attalea phalerata*

Locus (GenBankID)	Repeat motif	Primer sequence (5' to 3')	Size (bp)	T_a	Ho	He	HWE	#Alleles
A103_3 (HM563062)	GT_9	F-CAATGCAAGAGACAAGCATAC R-GCACACTTGATGACATTTTATG	235-259	59.9	0.758	0.767	NS	8
A106_2 (HM563063)	CA_{11}	F-CATTTGGCATTCTTACACATTC R-CTTGGGGTGAACTGACTTTAC	158-178	60.8	0.751	0.743	NS	8
B101_4 (HM563064)	TC_{19}	F-CCTGGTCATCCGATTATTTCA R-TGTCGCCATTCTTTCGTTTAT	132-166	63.4	0.797	0.814	NS	17
B102_8 (HM563065)	GA_{21}	F-AGCACTAATGTGCATGTATGTG R-CCATTCCCTCTACAAGGATAAC	160-190	60.1	0.841	0.843	NS	17
B103_3 (HM563066)	TC_{17}	F-ATGCTGCTTGCGGTGTAG R-GAGGTATTGATGGGAGGAAGAC	192-232	62.7	0.836	0.871	NS	16
B121_4 (HM563067)	TC_{12}	F-CCTGGAGCATCAATGGAC R-TCCGAGAACCCTAAACCTG	121-147	61.7	0.869	0.845	NS	12
C5_4 (HM563068)	GTT_9	F-AAGATGACCGTAGCATTAACAG R-TCCCATGTTTTCTTTAGTCTTC	251-275	59.7	0.646	0.681	NS	9
C11_7 (HM563069)	CAA_9	F-AGTCGTGAACTCTACCACTTTC R-TGTTGCCCTTCAGATATAGATC	214-238	58.9	0.633	0.649	NS	9
C122_3! (HM563070)	CAA_8	F-TCCTCCACCTCCAATGGTAG R-TTCGACATGACGAGAACGTC	257-275	63.8	0.541	0.65	***+	8
D2_1! (HM563071)	$ATCT_7$	F-CTGTCCTGCACAAGAAGTTGA R-AAGTTGGCGCATATCAATGAC	200-217	63	0.688	0.463	***+	3
D3_2! (HM563072)	$ATCT_5$	F-ACAGGTGTGGTTCAATCAAT R-GGAGGGATAGACAAAGGAAG	228-283	59.3	0.26	0.61	***+	10

D106_3 (HM563073)	$AGAT_7$	F ACCACCCCATCACAAAAG R GGACCATTTCAGCCAGAG	171-215	60.8	0.557	0.611	NS	10
D110_8! (HM563074)	$ATCT_7$	F-GCAGTGGTATGCCTGTATAGTG R-CTAGCACACATGCACACATG	163-201	61	0.435	0.74	***+	17
D124_5 (HM563075)	$ATCT_{10}$	F-GGTGGTGATTGAACTGAACTC R-GCTGATGCTTGCTGACAG	233-299	61	0.891	0.881	NS	25

T_a = annealing temperature; Ho = observed heterozygosity, He= expected heterozygosity, HWE= Hardy-Weinberg Equilibrium test, NS indicates not significant and ***indicates significant p-value <0.0000001; + indicates significant presence of null-alleles; ! indicates primer pairs that exhibited significant deviations from HWE and significant presence of null-allelles.

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